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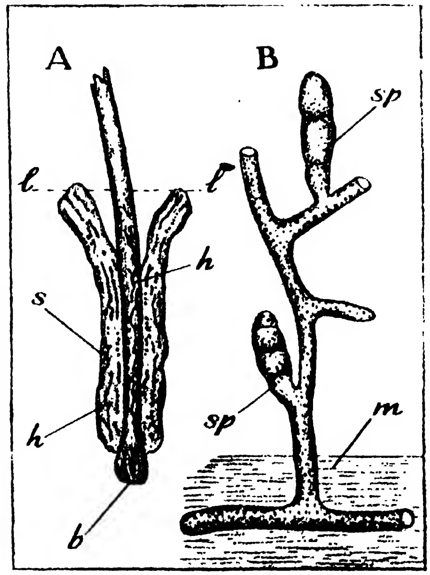

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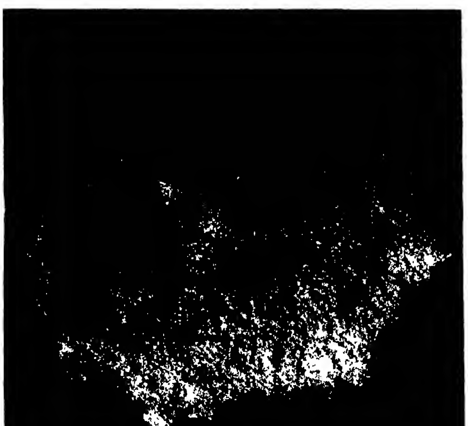

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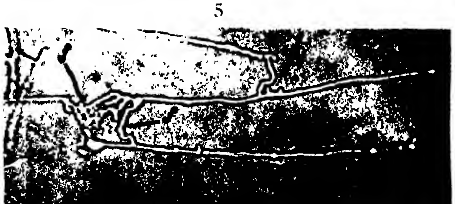

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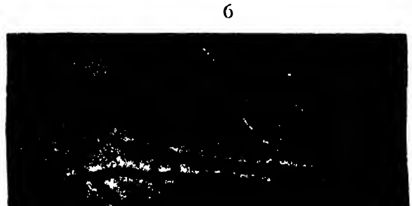

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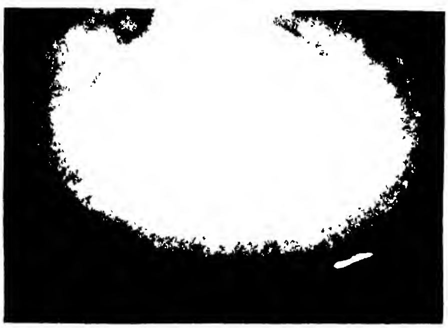

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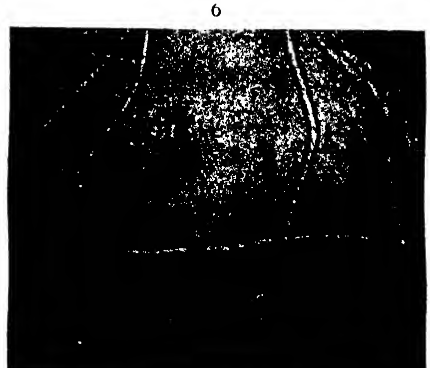

Microsporon audouini

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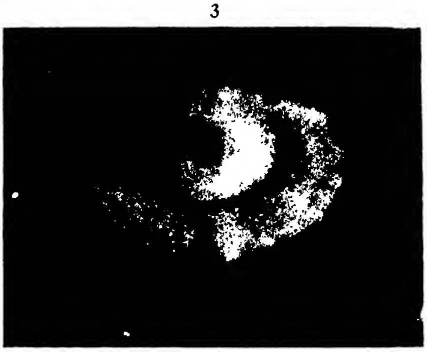

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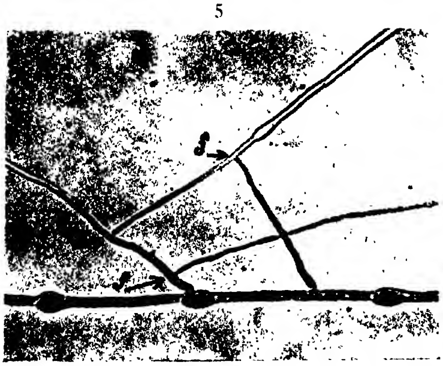

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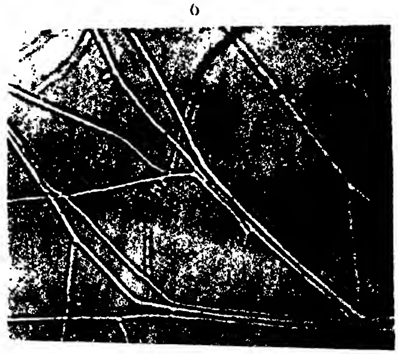

Microsporon lanosum

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HYPHAL FUSIONS IN DERMATOPHYTES[1]

BY A. M. DAVIDSON[2], ELEANOR S. DOWDING[3] AND A. H. R. BULLER[4]

Abstract

Hyphal fusions have been recognized as an important character of dermatophytes.

In *Microsporon audouini*, *M. lanosum*, and *Trichophyton gypseum* hyphal fusions: (1) are formed between hyphae of one and the same mycelium isolated from a single patient, (2) are formed between any two mycelia of the same species isolated from two different patients, and (3) are *not* formed between a mycelium of one species and a mycelium of another species.

The occurrence or non-occurrence of hyphal fusions between hyphae of two mycelia of different origin may be applied as a criterion for identifying species of dermatophytes whose specific nature is uncertain.

I. Introduction

As dermatologists are well aware, the taxonomy of the fungi which cause diseases of the skin is in a confused state. This is due in part to the fact that dermatophytes in general are Fungi Imperfecti which yield no perfect fruit-bodies, and in part to the inconstancy in the characters of the mycelium when grown for a considerable period of time. Hitherto, species have been distinguished from one another (1) by macroscopic appearance when grown on Sabouraud's medium and (2) by the spores and swellings which may be observed upon their individual hyphae (5, 8).

In 1931, Buller (3), in the fourth volume of his *Researches on Fungi*, called attention to the prevalence and physiological importance of the hyphal fusions in the mycelia of Ascomycetes, Basidiomycetes, and Fungi Imperfecti. He pointed out that, in many species of fungi, hyphal fusions may take place between two hyphae of one and the same mycelium derived from a single spore, or between a hypha derived from one monosporous mycelium and a hypha derived from another monosporous mycelium. Furthermore, in discussing the various functions of hyphal fusions, he showed that the formation of hyphal fusions need not have, and indeed very frequently does not have, any connection with sexual phenomena.

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Contribution from the University of Manitoba, Winnipeg, Canada. This work was made possible by a grant from the Banting Research Foundation.
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In 1924, Buller (2) paired the diploid mycelium of *Panus stypticus** physiological form *luminescens* of North America with *P. stypticus* p.f. *non-luminescens* of England and Europe, and observed that hyphal fusions are formed between the two mycelia. He regarded the occurrence of these hyphal fusions as supporting the view that the two physiological forms of *P. stypticus*, which occur on opposite sides of the Atlantic Ocean, belong to one and the same species. Subsequent work, as yet unpublished, carried out by him with the assistance of Miss Ruth Macrae, has confirmed his conclusion; for it has been found that, when a haploid mycelium derived from a single spore of *P. stypticus* p.f. *luminescens* is paired with a haploid mycelium derived from a single spore of *P. stypticus* p.f. *non-luminescens*, a diploid clamp-connection-bearing mycelium results.

Buller's observations on hyphal fusions in fungi generally have suggested the following questions: are hyphal fusions present in dermatophytes; and, if so, of what value are they for diagnostic purposes? An attempt to answer these questions has been undertaken by the authors and the results of their investigations upon *Microsporon audouini*, *M. lanosum*, and *Trichophyton gypseum* are recorded in this paper.†

II. Microsporon audouini

(a) Methods

Natural Occurrence.—Of the 43 patients suffering from tinea capitis, who have come to the notice of the authors during the last six months, in more than half of them (65%) the condition was due to an infection by *Microsporon audouini* Gruby (Plate I, Fig. 1). Evidently, this fungus is the commonest cause of ringworm of the scalp in Winnipeg. This conclusion accords well with that of Adamson (1) who, in 1895, recorded that the frequency of ringworm cases in England due to *Microsporon audouini* was 80-90%.

Selection of Hairs.—Infected hairs from the head of a patient suffering from tinea capitis were selected by means of a water-cooled ultra-violet light apparatus equipped with a Wood's filter. This method was first introduced by Vigne (7). In the light obtained from the water-cooled lamp with the filter, the infected hairs show a green fluorescence and thus they can readily be distinguished from the healthy hairs.

Culture Media.—The infected hairs were planted in hanging drops of Sabouraud's medium or, less frequently, of sterile water. As was to be expected, the growth of the fungus was not so rapid in water as in Sabouraud's medium.

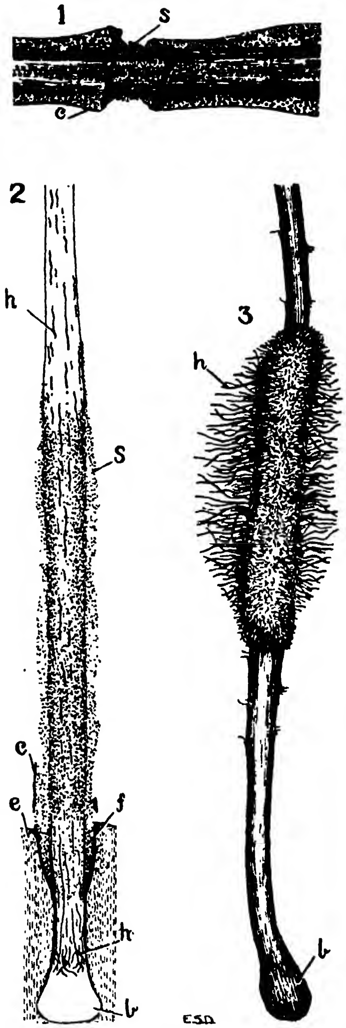
Two different modifications of Sabouraud's medium were employed. They did not contain the special maltose or peptone recommended by Sabouraud, but both gave satisfactory results. (1) Forty grams of pure commercial

**Panus stypticus is a wood-destroying gill-fungus. Its small bracket-like fruit-bodies occur on stumps of the birch. In the North American form both the mycelium and the fruit-bodies give out light. The English and European form is non-luminous in all stages of its development.*

†*The authors gratefully acknowledge their indebtedness to Dr. Howard Fox and Dr. E. Muskatblit of the Department of Dermatology of New York University for carefully verifying their determination of the three species of fungi here named.*

maltose, 10 gm. of standardized bacto-peptone, and 18 gm. of shredded agar-agar were dissolved in one litre of distilled water in an autoclave. The mixture was then filtered through paper and poured into containers. It was finally sterilized by allowing the pressure in the autoclave to rise slowly to 10 lb. upon three successive days. (2) An American commercial preparation of Sabouraud's medium manufactured by "Difco" was used. This contained 10 parts of bacto-peptone, 40 parts of bacto-dextrose, and 15 parts of bacto-agar. To make up this preparation, 65 gm. of the mixture was boiled with a litre of water, dispensed into containers, and sterilized in the same manner as before.

Culture Method.—Hairs infected with *M. audouini* become so packed with fungal spores beneath the cuticle (Text-fig. 1) that the cuticle is soon ruptured and peels off, thus exposing the sheath of spores (Text-fig. 2). The spore-sheath extends from below the level of the scalp to a distance of several millimetres above the follicle. When an infected hair is planted in a hanging drop of Sabouraud's medium or of water, the spores making up the sheath commence to germinate after about eight hours. Infected hairs were sown in flasks of Sabouraud's medium, and after 24 hr. the base of each hair could be seen with the naked eye to be surrounded by a white halo made up of hyphae radiating out from the sheath (Text-fig. 3 and Plate I, Fig. 2). Mycelia obtained from infected hairs were stored in test tubes and Erlenmeyer flasks containing Sabouraud's medium.



TEXT-FIGS. 1–3. Hairs from scalp infected with *Microsporon audouini;* magnification, 43. FIG. 1. Portion of a hair with its cuticle ruptured but still retained: *c*, cuticle of hair; *s*, fungal spores. FIG. 2. A hair in its follicle, after the loss of the cuticle: *b*, bulb of the hair; *c*, cuticle of the hair; *e*, epithelial tissue of the scalp; *f*, follicular cavity; *h*, fungal hyphae; and *s*, fungal spores. FIG. 3. A hair which had been in Sabouraud's medium a few days. The fungal spores have produced hyphae which have grown out into the nutrient medium: *b*, bulb of the hair; *h*, fungal hyphae.

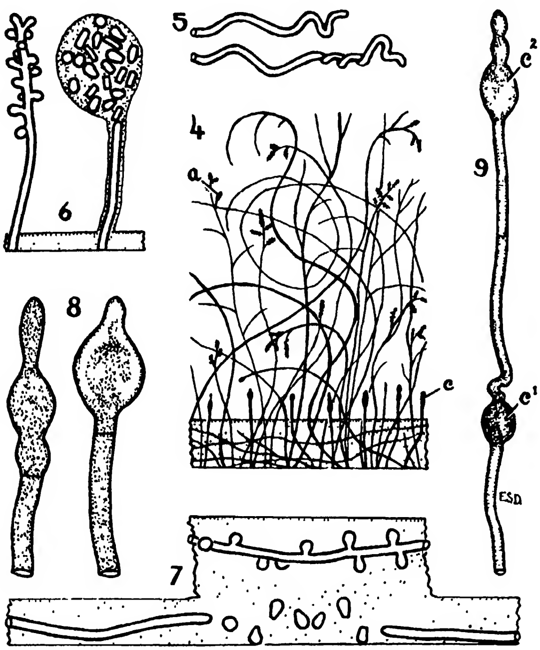
(b) THE MYCELIUM

The Young Mycelium.—The hyphae of *M. audouini* grow out from an infected hair or from a stock-culture inoculum into the medium to form a mycelium which remains in a vegetative condition for the first three or four days.

The hyphae grow at the rate of 0.25 to 0.5 mm. per day. The width of the hyphae varies from 3 to 6 μ. The smaller hyphae are often sinuous or wavy in outline and sometimes are spirally twisted into corkscrew shapes (Text-

fig. 5). When the mycelium is about two days old, one or two hyphal fusions may be observed. A detailed description of hyphal fusions will be given later.

After the mycelium has been growing in a hanging drop of Sabouraud's medium for three or four days, some of the hyphae commence to grow out of the medium into the air. The aerial mycelium developed in flask cultures forms a nap or down above the surface of the culture medium. As the aerial hyphae are not very long, the nap is close and extends only about a quarter of a millimetre above the level of the medium (Text-fig. 4 and Plate I, Fig. 3). It is these aerial hyphae which produce the spores.



TEXT-FIGS. 4–9. Aerial hyphae and spores of *Microsporon audouini;* the agar medium in Figs. 4, 6, and 7 has been stippled. Magnification: Fig. 4, 45; Figs. 5–9, 400. FIG. 4. Diagram of a lateral view of an aerial mycelium as it appears when a transverse section is made through the agar on which the fungus is growing: *a*, aleuriospores; *c*, chlamydospore. FIG. 5. Aerial hyphae, showing spiral twisting. FIG. 6. Aleuriospores borne upon aerial hyphae. The spores to the right have become detached and are floating in the drop of water collected on the conidiophore. FIG. 7. Hyphae with aleuriospores submerged in the medium. Part of the lower hypha has disintegrated and freed the spores. FIG. 8. Chlamydospores. FIG. 9. A hypha which has continued its growth through and beyond the chlamydospore c^1 and has produced a second chlamydospore c^2.

Aleuriospores.—When the mycelium has been growing in a hanging drop of Sabouraud's medium for about five days, some of the aerial branches (and to a less extent some of the submerged ones) form lateral conidia or aleuriospores (Text-fig. 4, *a*). These aerial conidia break off from the conidiophores and the conidiophores themselves segment. All the free cells which are thus formed frequently collect in liquid drops which appear on the aerial hyphae (Text-fig. 6). When the aleuriospores are borne on submerged hyphae, they are sometimes set free by the disintegration of the main hypha (Text-fig. 7).

Chlamydospores.—When the mycelium is about two weeks old, a large number of hyphae grow out into the air a short distance from the surface of the culture medium. These aerial hyphae are less than a quarter of the length of the aerial hyphae which form the aleuriospores and, at their apices, they form lemon-shaped swellings termed chlamydospores (Text-fig. 4, *c* and Plate I, Fig. 4). In spite of the implication of their name, the chlamydospores are not thick-walled and they have

never been observed to become detached. For the present, therefore, they cannot be regarded as organs of dissemination. Growth is sometimes renewed at the base of the chlamydospores: a hypha grows through the old swelling, emerges at the swelling's apex, and then continues its growth in the culture medium (Text-fig. 9).

Hyphal Fusions.—Hyphal fusions were observed: (1) between two hyphae of one and the same mycelium derived from a single hair; and (2) between two hyphae which originated from two mycelia derived from hairs of different patients.

(1) A hair of patient A was placed in Sabouraud's medium in a flask and a mycelium of *M. audouini* was obtained from it. This mycelium was grown in a conical flask for three months. At the end of this time, a small pin-head mass of the aerial mycelium was removed from the flask and was set in the middle of a hanging drop of Sabouraud's medium in a van-Tieghem cell the bottom of which was covered with a shallow layer of sterile water. In the course of about two days the hyphae grew out from the inoculum into the culture medium. The hanging drop was about 3 mm. in diameter. The mycelium grew out radially and, in the course of three days, attained a diameter equal to that of the drop. It then pushed out beyond the drop into the film of water which had been formed by condensation on the cover-glass.

Five days after inoculation, hyphal fusions were observed both within the culture medium and in the film of water surrounding it. The fusions within the medium were relatively few and were observed only after careful search. On the other hand, the fusions between hyphae in the film of moisture were very numerous and could be determined with ease. In one particular part of the mycelium growing in the water-film, having a length of 1 mm. and a breadth of 0.2 mm., nine hyphal fusions had been established. Hyphal fusions, seen in the area of the mycelium to which reference has just been made, are shown in Text-figs. 10, 11 and 12.

From an examination of Text-figs. 10, 11 and 12 it appears that all the fusions there illustrated were formed between the end of one hypha and the lateral wall of another hypha. This type of hyphal fusion has been described and illustrated by Buller (3).

From an examination of the photograph of the mycelium of *M. audouini* in Plate I, Fig. 5, it can be seen that the hyphal fusion there illustrated probably was formed as follows. Two hyphae happened to be parallel and then, at a point where the two hyphae were close together, they sent out short peg-like protuberances which grew directly towards each other and fused apically. In Plate I, Fig. 5, near the middle of the uppermost pair of hyphae, two of these peg-like protuberances can be seen before they have come into contact.

In a mycelium growing out into the water-film around a hanging drop of culture medium, more and more hyphal fusions were formed as the mycelium extended in area. Finally, at the end of about two weeks after inoculation, by which time the culture medium had become exhausted, the number of hyphal fusions was often 100 or more. It seems probable that the formation of hyphal fusions is favored by starvation.

Similar observations to those just recorded were made with each of two mycelia of *M. audouini* isolated from single hairs of two other patients, B and C, also suffering from tinea capitis.

(2) Mycelia of *M. audouini* derived from the patients A, B and C were now grown together in pairs. The pairs were A-B, A-C, and B-C. The three pairs were established in as many hanging drops of Sabouraud's medium which were similar to those described above. To make a pair, a pin-head mass of the aerial hyphae of one of the mycelia was set near the middle of the drop and then a similar mass from another mycelium was set in the drop 1-2 mm. from the first mass (Text-fig. 13, *a*).

The two inocula in each hanging drop grew out into the medium in the usual way (Text-fig. 13, *b*) and then out into the film of water on the cover-glass. The hyphae of the two mycelia along the line of contact soon came to cross one another at various angles (Fig. 13, *c*).

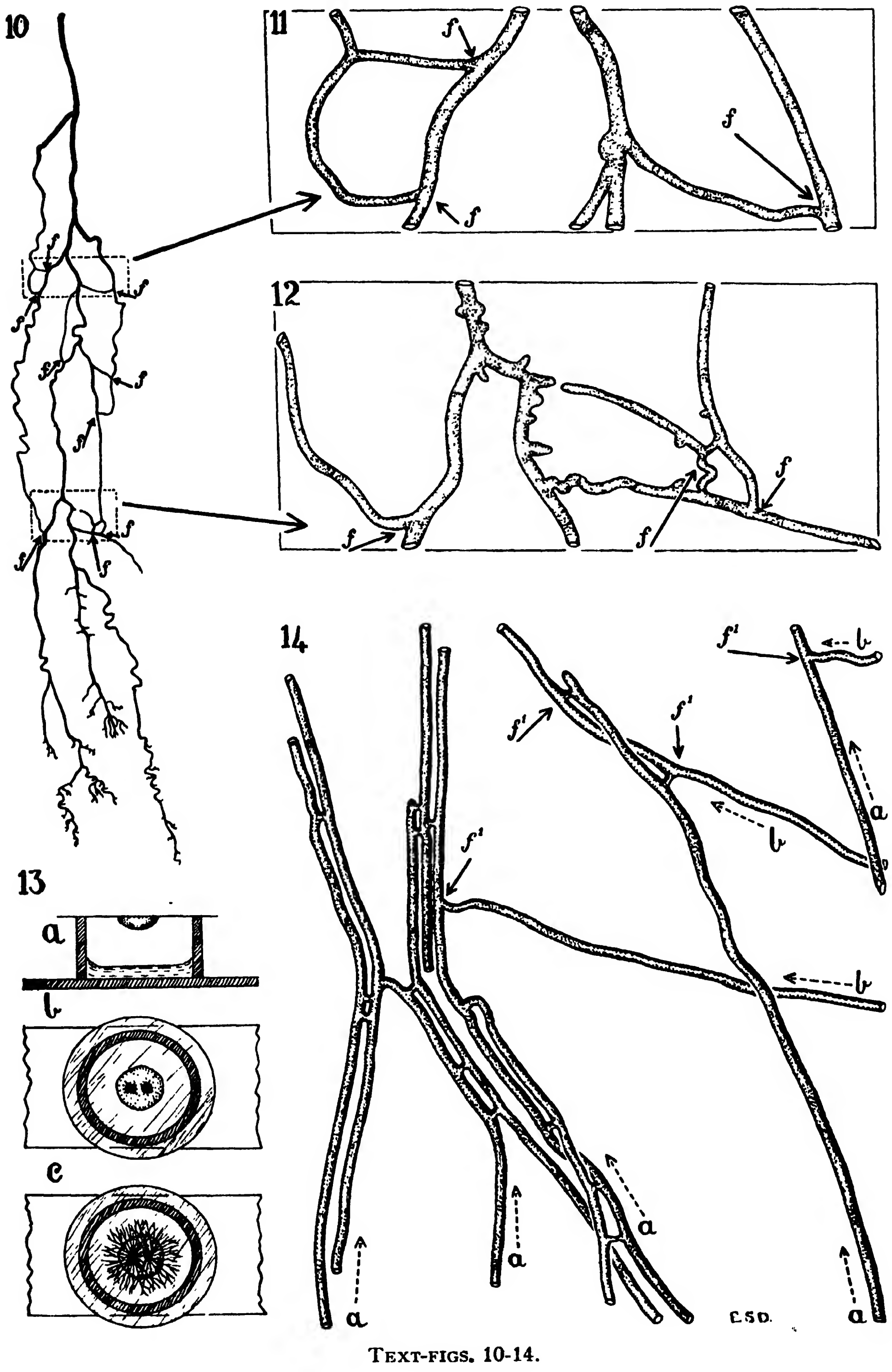
In each of the three hanging drops, hyphal fusions were sought for between a hypha derived from one mycelium and a hypha derived from the other mycelium, and they were readily found in the film of water outside the medium (Text-fig. 14 and Plate I, Fig. 6). Scores of such hyphal fusions between the two mycelia in each of the three pairs were observed. Convincing evidence was thus obtained that *hyphal fusions readily take place between two mycelia of Microsporon audouini, one derived from a hair of one patient and the other derived from a hair of another patient.*

III. Microsporon lanosum

(a) Methods

Natural Occurrence.—Microsporon lanosum Sab. is a parasite of the hair and the skin. On the scalp it results in crusted lesions, and on the glabrous skin it forms circular, red, scaly lesions (Plate II, Fig. 1). At Winnipeg, after *M. audouini*, *M. lanosum* is the commonest cause of ringworm of the scalp. Seven of the 56 children with ringworm who were under observation during the last six months were infected with *M. lanosum*.

TEXT-FIGS. 10–14. Hyphal fusions in the mycelium of *Microsporon audouini*. FIG. 10. Some of the peripheral hyphae of a mycelium which had been growing in a hanging drop of Sabouraud's medium for a month. The hyphae have grown out of the medium and are in the water condensed on the cover-slip: *f f f*, fusions between hyphae of the same mycelium. Magnification, 70. FIGS. 11 and 12. Portions of Fig. 10, drawn more highly magnified: *f f f*, fusions between hyphae of the same mycelium. Magnification, 400. FIG. 13. Diagrams to show the materials and methods used to determine whether or not hyphal fusions are formed between different mycelia: *a*, inocula of the two mycelia to be tested have been planted side by side in a hanging drop of nutrient medium in a van-Tieghem cell; *b*, the inocula have commenced to grow; *c*, the two mycelia from the inocula have grown out of the medium into the water condensed on the cover-glass where they have met and crossed each other. It is in the parts of the culture where the two mycelia have crossed each other in the water-film outside the culture medium that fusions can most readily be found. Natural size. FIG. 14. Hyphal fusions between two mycelia of *M. audouini*. Two mycelia, *a a a* and *b b b*, obtained from patients A and B respectively, have been grown together in a hanging drop of culture medium (*cf.* Fig. 13) for two weeks. The arrows with broken shafts by the letters *a a a* and *b b b* indicate the direction of growth of the hyphae. The hyphae of the mycelium *a a a* have met with, and have fused with, the hyphae of the mycelium *b b b* at four places each of which is indicated by an arrow and the letter f^1. Magnification, 400.



TEXT-FIGS. 10-14.

Sartory (6) considers that *M. lanosum* is the most common dermatophyte of animal origin.

Selection of Infected Tissue.—The mycelium was obtained either from infected hairs or from infected skin. The infected hairs were selected by means of the water-cooled ultra-violet light with Wood's filter attachment. Infected hairs examined in this light gave out a green fluorescence. The infected skin which was used consisted of epidermal scales selected from the margins of the skin lesions. This tissue was always penetrated by fungal hyphae (Text-fig. 15).

Culture Media.—The culture media employed for growing *M. lanosum* were the two modifications of Sabouraud's medium already described.

Culture Method.—Hairs or epidermal scales infected with *M. lanosum* were placed in hanging drops of Sabouraud's medium. A day or two after an infected hair had been placed in a hanging drop, a mycelium could be seen growing out from the region of the hair that had previously been observed to be covered by a sheath of fungal spores. A day or two after an infected epidermal scale had been planted in a hanging drop, a mycelium could be seen growing out from every part of the surface of the scale. The mycelium was allowed to continue its growth for a week or two, after which it was transferred to flasks or test tubes of Sabouraud's medium.

Similar morphologically and characteristic of *M. lanosum* were: (1) five mycelia obtained from as many different patients suffering from tinea capitis and tinea corporis; and (2) two mycelia obtained from the same patient, one mycelium taken from a lesion on the scalp and the other taken from a lesion on the glabrous skin of the body.

(b) The Mycelium

The Young Mycelium.—The mycelium of *M. lanosum* grew from the infected hairs or epidermal scales into hanging drops of Sabouraud's medium at the rate of 0.3-0.5 mm. per day. Within about four days after tissue infected with *M. lanosum* has been placed in the culture medium, large numbers of aerial hyphae grow out from the medium down into the air in the van-Tieghem cell and there attain a length of several millimetres. The aerial hyphae are inclined to twine around each other so as to form twisted strands (Text-fig. 16). The profuse growth of aerial mycelium characteristic of *M. lanosum* may be seen not only in van-Tieghem cells but also in test tubes and flasks, and it gives the culture a deep, white, felted covering or "duvet" (Plate II, Fig. 3).

Spindles.—After about a week of growth either in hanging drops or in test tubes or flasks, short aerial hyphae produce spindle-shaped multilocular spores called "spindles", "fuseaux", or "macroconidia" (Text-figs. 16 *s*, 19 and Plate II, Figs. 2, 4). The spindles can be obtained by tearing away the web of long aerial hyphae from a culture with a needle and then scraping the surface of the agar. The spindles adhere to the needle. By no means are they always present in *M. lanosum* cultures, for they were observed in only five of twenty-five or more hanging-drop cultures and in only two of five or more cultures growing in flasks. It was not until the two cultures in flasks had been kept for about four months that they began to form spindles, and two months later no spindles could be found in either of them.

Spindles (Text-fig. 19 and Plate II, Figs. 2, 4) are developed aerially. They may arise singly or be borne in clusters upon a branched hypha. Usually they are slender and attenuated at the apex, and sometimes they end in a terminal bristle. The spore-coat bears small wart-like protuberances on its outer surface. The cavity is divided transversely into about nine segments (Fig. 19).

As a mycelium develops, the form of its spindles may change. One particular mycelium, when four months old, possessed small blunt-ended spindles made up of six or even fewer segments, while, two months later, it had begun to form large pointed spindles made up of about nine or more segments. A second mycelium possessed normal spindles when it was three weeks old but, after three more months, the spindles were small and degenerate and possessed no septa. This variation in the form of spores has been noticed by other authors (4, 8), and it is noteworthy because the classification of some dermatophytes is largely based on the character of the spores.

Chlamydospores.—When the mycelium has been growing about three weeks in a flask or test tube, terminal chlamydospores are sometimes formed. These chlamydospores are similar to those of *M. audouini* but are not so abundant.

Conidia.—Some of the longer aerial hyphae may become divided into short segments by transverse walls (Text-figs. 16 *c*, 17, and 18). These segments become detached from the hyphae and act as conidia. They swell in water and become irregular in shape.

Intercalary Swellings.—The older submerged hyphae of *M. lanosum* become swollen into oval nodes at intervals along their length (Text-fig. 21, *i.s.*).

Hyphal Fusions.—Hyphal fusions were observed: (1) between two hyphae of one and the same mycelium derived from a single hair; and (2) between two hyphae which originated from two mycelia derived from hairs of different patients.

(1) A hair from patient D was placed in Sabouraud's medium in a flask and a mycelium of *M. lanosum* was obtained from it. This mycelium was grown in the flask for upwards of three months. At the end of this time, a small pin-head mass of the aerial mycelium was removed from the flask and set in the middle of a hanging drop of Sabouraud's medium in a van-Tieghem cell as used in a similar experiment with *M. audouini*. In the course of about two days, the hyphae grew out from the inoculum into the culture medium. The mycelium grew radially and, in the course of three days, attained a diameter equal to that of the drop. It then pushed out beyond the drop into the film of water which had been formed by condensation on the cover-glass.

Five days after inoculation, large numbers of hyphal fusions were observed both within the culture medium and in the film of water surrounding it (Text-figs. 20, 21 and Plate II, Fig. 5).

Observations similar to those just recorded were made with each of two mycelia of *M. lanosum* isolated from single hairs from two other patients, patients E and F, also suffering from tinea capitis and tinea corporis.

(2) Mycelia of *M. lanosum* derived from the patients D, E and F were now grown together in pairs. The pairs, which were D-E, D-F, and E-F, were established in hanging drops of Sabouraud's medium. To make a pair, a

pin-head mass of the aerial hyphae of one of the mycelia was set near the middle of the drop and then a similar mass from another mycelium was set in the drop 1-2 mm. from the first mass (Text-fig. 13, *a*).

The two inocula in each hanging drop began to grow into the medium after the first or second day (Text-fig. 13, *b*) and then into the film of water on the cover-glass. About ten days after inoculation, the hyphae of the two mycelia had come into contact and had commenced to cross each other at various angles (Text-fig. 13, *c*).

In each of the three hanging drops; hyphal fusions were sought for between a hypha derived from one mycelium and a hypha derived from the other mycelium, and they were readily found in the film of water outside the medium (Plate II, Fig. 6). Large numbers of such hyphal fusions between the two mycelia in each of the three pairs were observed. These observations, together with identical results obtained from a repetition of the experiments, afford convincing evidence that *hyphal fusions readily take place between two mycelia of Microsporon lanosum, one derived from the tissue of one patient and the other derived from the tissue of another patient.*

IV. Trichophyton gypseum

(a) Methods

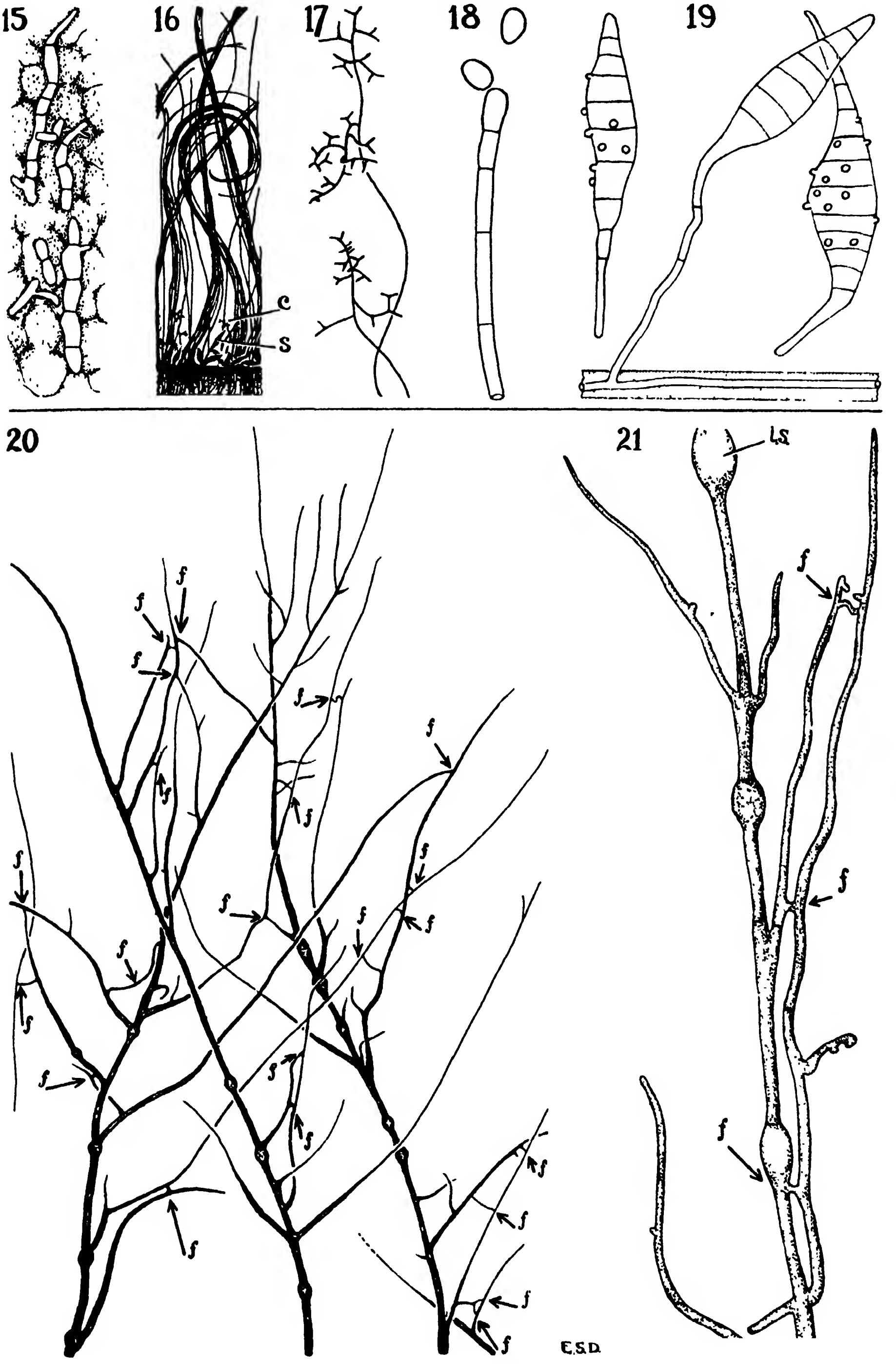
Natural Occurrence.—During the last six months, the authors examined twelve adult male patients with fungal diseases, and three of the twelve patients were found to be suffering from sycosis parasitaria (tinea barbae). *Trichophyton gypseum* Bodin (= *T. asteroides* Sab.) was isolated from the beard-hairs of one of the three patients, patient G, who is shown in Plate III, Fig. 1.

In patient G, the hairs of the beard area, particularly on the upper lip, were infected. An inflammatory reaction had been set up and pus was exuding from the hair follicles. Microscopical examination showed that the shaft and follicular sheath of each diseased hair had been penetrated by fungal hyphae (Plate III, Fig. 2, A).

Selection of Hairs.—The faces of patients with sycosis parasitaria were examined with the ultra-violet light apparatus, but none of the diseased beard-hairs gave out a green fluorescence. Ultra-violet light was therefore of no assistance in selecting hairs infected with *T. gypseum*. To obtain a hair which would give rise to a mycelium, it was necessary to remove large numbers of hairs from the diseased areas and place them upon Sabouraud's medium.

Culture Medium and Culture Method.—The culture media employed for

Text-figs. 15–21. The mycelium and spores of *Microsporon lanosum*. Fig. 15. A piece of an epidermal scale from patient D, penetrated by fungal hyphae. Magnification, 400. Fig. 16. A diagram of part of an aerial mycelium produced upon Sabouraud's medium, seen in lateral view: at the base, the culture medium; *c*, conidiophore; *s*, spindle. Magnification, 42. Fig. 17. Conidiophores and conidia. Magnification, 70. Fig. 18. Conidiophore and conidia, more highly magnified. Magnification, 400. Fig. 19. Spindles. Magnification, 400. Fig. 20. Mycelium from patient F, grown in a hanging drop of Sabouraud's medium for ten days: *f f f*, numerous hyphal fusions. Magnification 70. Fig. 21. Mycelium from patient E, grown in a hanging drop of Sabouraud's medium for ten days: *f f f*, hyphal fusions; *i.s.*, one of three intercalary swellings on a main hypha. Magnification, 400.



TEXT-FIGS. 15-21.

growing *T. gypseum* were the two modifications of Sabouraud's medium already described.

Beard-hairs infected with *T. gypseum* were placed in hanging drops of Sabouraud's medium. After a day or two some of the hairs had given rise to a mycelium. All of the mycelia were contaminated with bacteria from the pus adhering to the hair, so that it was necessary to separate the fungus from the bacteria. This was accomplished by transferring with a sterile needle a pin-head mass of aerial mycelium from the hanging drop to a flask of culture medium.

(b) The Mycelium

Spindles.—The mycelium of *T. gypseum* from patient G was allowed to grow in the flask for three weeks. It attained a diameter of a few centimetres and produced a dense, dead-white, aerial mass of hyphae. When a portion of the aerial mycelium was pulled away with a needle and examined microscopically it was found to contain a number of spindles of characteristic appearance. They were club-shaped with rounded ends, were about 20 μ long and 5 μ wide, and possessed two or three cross-walls (Plate III, Fig. 2, B).

The aerial mycelium, when three weeks old, in addition to spindles contained many hyphae that were twisted into close spirals.

Aleuriospores.—When the mycelium of *T. gypseum* had been in culture nearly a month, the spindles were no longer to be seen and the aerial hyphae were observed to be producing large numbers of aleuriospores (Plate III, Fig. 4). The aleuriospores of this species, unlike those of *Microsporon audouini*, tend to be constricted at their base. They are arranged laterally on the hyphae, and, when they are crowded together, the inflorescence has the appearance of a bunch of grapes.

By the time the culture was about a month old, the contour of the mycelium had become definitely star-shaped.

Chlamydospores.—As the medium upon which the fungus was growing became progressively exhausted, the texture of the surface of the mycelium became granular and plaster-like (Plate III, Fig. 3). Microscopical examination showed that this granular appearance was due to the aerial hyphae having swelled up at intervals along their length so as to form chains of intercalary chlamydospores. The chlamydospores were spherical and the largest were 14 μ in diameter. The aerial hyphae of a four-months-old culture was made up almost entirely of chlamydospores.

When transfers of the fungus were made from an exhausted medium to a fresh medium, spindles and aleuriospores were again obtained.

Pleomorphism.—When a culture of *T. gypseum* was about six months old, a white downy growth of sterile aerial hyphae commenced to grow over its surface. This change is known in the literature as "pleomorphism." Pleomorphism is characteristic of dermatophytes and, according to our present knowledge, it is an irreversible change.

Hyphal Fusions.—Hyphal fusions were observed: (1) between two hyphae of the same mycelium derived from a single hair; and (2) between two hyphae which originated from two mycelia derived from hairs of different patients.

(1) A hair from patient G was placed in Sabouraud's medium in a flask and a mycelium of *T. gypseum* was obtained from it. When the mycelium had been growing in the flask for four months, a small pin-head mass of the aerial mycelium was removed from the flask and set in the middle of a hanging drop of Sabouraud's medium in a van-Tieghem cell, the bottom of which was covered with a shallow layer of sterile water. In the course of two days, the hyphae grew out from the inoculum into the culture medium. It then attained a diameter equal to that of the drop, and then pushed out beyond the drop into the film of water which had been formed by condensation on the cover-glass.

About a week after inoculation, a few hyphal fusions were found within the drop of culture medium, and many in the film of water surrounding it (Plate III, Fig. 5).

Observations similar to those just recorded were made with a mycelium of *T. gypseum* obtained from another patient, H, who was also suffering from sycosis parasitaria. The material from patient H was obtained in New York by Dr. Muskatblit.

(2) The mycelium of *T. gypseum* derived from patient G in Winnipeg was then grown with the mycelium of *T. gypseum* sent from New York and derived from patient H. The pair was established in a hanging drop of Sabouraud's medium. To make the pair, a pin-head mass of the aerial hyphae of mycelium G was set near the middle of the drop and then a similar mass of mycelium H was set in the drop 1-2 mm. from the first mass (Fig. 13, *a*).

After the two mycelia had crossed each other, hyphal fusions were sought for between a hypha derived from one mycelium and a hypha derived from the other. After 13 days, seven or eight hyphal fusions between mycelia G and H were discovered along the margin of the medium (Plate III, Fig. 6). This experiment goes to show that *hyphal fusions readily take place between two mycelia of Trichophyton gypseum, one derived from a hair of one patient and the other from a hair of another patient,* even though these patients are from widely separated geographical areas.

V. Pairings between Mycelia of Different Species

(a) Microsporon audouini with M. lanosum

Six mycelia were employed. Three of them were *M. audouini* obtained from patients A, B and C, and the other three were *M. lanosum* obtained from patients D, E and F.

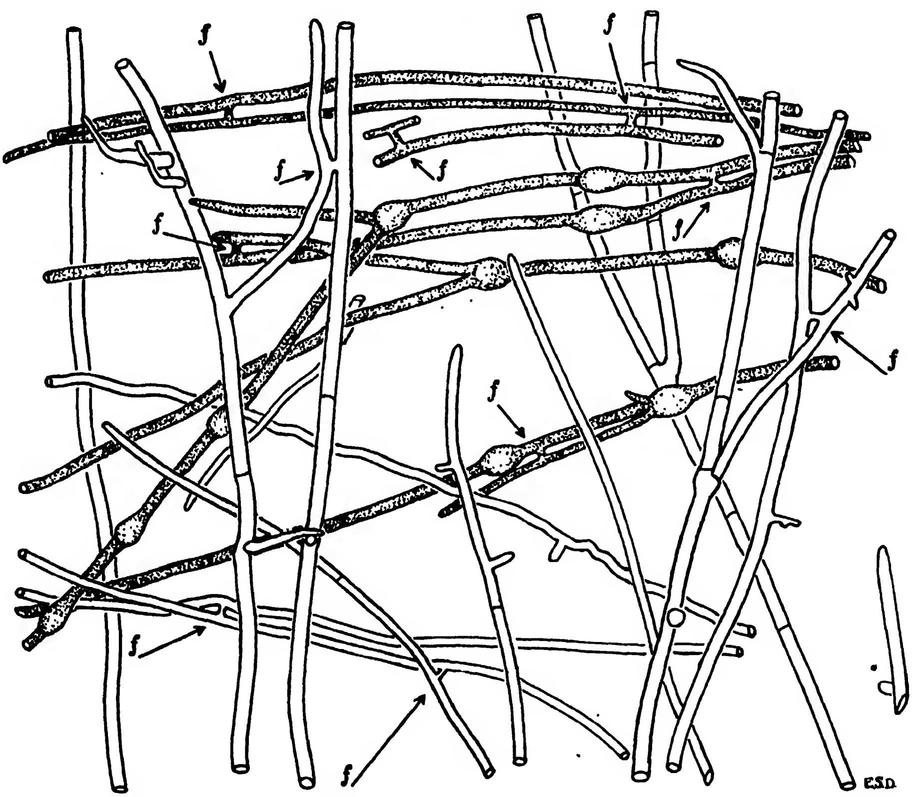
The mycelia were grown in hanging drops in pairs in the manner already described. Pairings between the mycelia of *M. audouini* and the mycelia of *M. lanosum* were made in all possible ways. Two hanging-drop cultures were made of each pair. Table I embodies the results obtained.

The appearance of the paired mycelia B-E in experiment No. 5 after one month is shown in Text-fig. 22. The hyphae of *M. lanosum* are characterized by intercalary swellings, while those of *M. audouini* can be distinguished by a slight tendency to waviness. In Text-fig. 22, to aid the eye in distinguishing the two mycelia, the mycelium of *M. lanosum* has been shaded while that of

TABLE I

Results of pairing mycelia of *Microsporon audouini* with mycelia of *M. lanosum*

Experiment No.	Pairs of mycelia	Date of inoculation	Result of examinations of the preparations made at intervals up to May 22
1	A – D	April 25	In each of the nine pairings fusions were observed between hyphae of the same mycelium but never between hyphae belonging to the two different mycelia
2	A – E	April 25	
3	A – F	April 25	
4	B – D	April 16	
5	B – E	April 25	
6	B – F	April 25	
7	C – D	April 25	
8	C – E	May 5	
9	C – F	May 5	



Text-fig. 22. Absence of hyphal fusions between the mycelia of two species of Microsporon. The mycelium of *Microsporon audouini* from patient *C*, left *unshaded*, and the mycelium of *Microsporon lanosum* from patient E, *shaded*, which have been growing side by side in a hanging drop of Sabouraud's medium for three weeks, have come into contact with one another, have become interlaced, but have *not fused* with one another. Hyphal fusions may be observed between two hyphae of *M. audouini* or between two hyphae of *M. lanosum* but not between a hypha of *M. audouini* and a hypha of *M. lanosum*: *f f f*, numerous hyphal fusions, but none of them between a hypha of *M. audouini* and a hypha of *M. lanosum*. Magnification, 400.

M. audouini has been left unshaded. It can be seen that there is an abundance of fusions between hyphae of the same species, but that there are no fusions between hyphae of different species.

As indicated in Table I, in all of the nine experiments, while fusions could be observed between hyphae belonging to one and the same mycelium of either *M. audouini* or *M. lanosum*, *no fusions could be observed between a hypha of M. audouini and a hypha of M. lanosum.*

(b) Trichophyton gypseum with Other Species of Dermatophytes

(1) *Trichophyton gypseum with Microsporon audouini.*—Three mycelia were employed. Two of them were *M. audouini* obtained from patients A and B and the other was *T. gypseum* obtained from patient G.

Two experiments were made. One hanging drop was inoculated with *T. gypseum* from patient G and *M. audouini* from patient A; and another hanging drop was inoculated with *T. gypseum* from patient G and *M. audouini* from patient B.

Three weeks after the two experiments had been set up, the hanging drops were examined microscopically and it was found that, although fusions could be observed between hyphae belonging to one and the same mycelium of either *T. gypseum* or *M. audouini*, *no fusions could be observed between a hypha of T. gypseum and a hypha of M. audouini.*

(2) *Trichophyton gypseum with Microsporon lanosum.*—Four mycelia were employed. Three of them were *M. lanosum* obtained from patients D, E and F, and the fourth was *T. gypseum* obtained from patient G.

The mycelia were grown in hanging drops in pairs in the manner already described. Pairings between the mycelia of *T. gypseum* and *M. lanosum* were made in all possible ways. Four hanging-drop cultures of each pair were set up.

Three weeks after the experiments had been started, the preparations were examined microscopically and it was found that, although fusions could be observed between hyphae belonging to one and the same mycelium of either *T. gypseum* or *M. lanosum*, *no fusions could be observed between a hypha of T. gypseum and a hypha of M. lanosum.*

(3) *Trichophyton gypseum with T. granulosum.*—Two mycelia were employed. One of them was *T. gypseum* obtained from patient G, and the other was *T. granulosum* Sab. obtained in New York by Dr. Muskatblit from patient I.

The mycelia were grown together in three hanging drops in the manner already described.

Three weeks after the experiments had been set up, the preparations were examined microscopically and it was found that, although fusions could be observed between hyphae belonging to one and the same mycelium of either *T. gypseum* or *T. granulosum*, *no fusions could be observed between a hypha of T. gypseum and a hypha of T. granulosum.*

T. granulosum is almost identical botanically with *T. gypseum*, and the two species are placed together by systematists (6) in the "Gypseum Group". That

hyphal fusions are not formed between these species is therefore of particular interest.

(4) *Trichophyton gypseum and Epidermophyton interdigitale.*—Two mycelia were employed. One was *T. gypseum* obtained from patient G, and the other was *E. interdigitale* Priestley obtained in New York by Dr. Muskatblit from patient J.

The mycelia were grown together in three hanging drops in the manner already described.

Three weeks after the experiments had been set up, the preparations were examined microscopically, and it was found that, although fusions could be observed between hyphae belonging to one and the same mycelium of either *T. gypseum* or *E. interdigitale, no fusions could be observed between a hypha of T. gypseum and a hypha of E. interdigitale.*

Cultures of *T. gypseum* and *E. interdigitale* on artificial media cannot be distinguished by experts, either by the naked eye or by a microscopic examination of the mycelia and their spores. They can be distinguished only clinically, *i.e.* by their parasitic behavior. *T. gypseum* attacks the beard-hairs, while *E. interdigitale* is restricted to the glabrous skin.

In spite of the very close resemblance of the two fungi, the lack of hyphal fusions between them in the experiment just recorded may be taken as evidence that they are indeed two different species.

VI. The Value of Hyphal Fusions for Identifying Species of Dermatophytes

It has been shown for the dermatophytes so far examined that: (1) hyphae belonging to the same mycelium fuse readily with one another; (2) when any two mycelia of different origin but of the same species are grown side by side, hyphal fusions are formed between hyphae of one mycelium and hyphae of the other; and (3) no hyphal fusions are formed between mycelia of different species.

In the light of the facts which have just been given, it is possible to apply a new method in the identification of dermatophytes. This method may be described as follows.

A mycelium of the fungus which one desires to identify is isolated from the diseased tissue of a patient and is grown in a culture medium. In the laboratory there must be stock cultures of a series of species of dermatophytes which have been properly identified. Then small pin-head masses of the unidentified mycelium are paired with similar masses of the mycelia of identified species in a series of hanging drops of culture medium suspended in as many van-Tieghem cells. The identified species chosen for the pairings are those which have produced in patients conditions clinically similar to those produced by the unidentified fungus. Let A, B, C, D and E be the identified species and X the unknown species used in the experiments. Then we have the following pairings: A-X, B-X, C-X, D-X, and E-X. At the end of about three weeks the cultures are examined with the microscope for hyphal fusions. Then it

will be found that hyphal fusions between the two mycelia in a van-Tieghem cell are present in one of the pairings but not in any of the others. From this result one may conclude that the species under investigation is identical with the one with which it forms hyphal fusions. If, for example, hyphal fusions between the two mycelia were found to be present in the pairing C-X but not in the pairings A-X, B-X, D-X, or E-X, such a result would indicate that the species X is identical with the species C.

By the method just described certain species of dermatophytes—those which form hyphal fusions—can be identified within a few weeks without the investigator waiting, often for months, for the characteristic spores to appear.

The presence or absence of hyphal fusions in pairing experiments has enabled the authors to determine the fungi responsible for the skin condition of (1) patient F and (2) patient H.

(1) Patient F, aged ten years, suffering from tinea corporis, showed scaling lesions one to two inches in diameter on the chest, back, and forehead. Each lesion was marked at the periphery by a circle of white vesicles. Examination of the child's body with the ultra-violet light and Wood's filter showed that occasional hairs on the lesions gave out a green fluorescence. Circular scaling lesions, such as those exhibited by patient F, may be caused by any one of several different dermatophytes.

A hair that was fluorescent in ultra-violet light was removed and stained with the gentian-violet stain combination for demonstrating fungi. An examination with the microscope revealed a mycelium within the hair and a mass of spores outside the hair. When epidermal scales from the lesions were treated with gentian violet, branching hyphae could be seen penetrating the tissue.

Infected epidermal scales were planted on Sabouraud's medium, and a mycelium appeared upon the medium two days later. The mycelium increased in size and took on a yellow color but remained perfectly sterile.

At the end of several weeks, the patient had been cured by iodine applications and had been discharged from the hospital, but the fungus which caused the disease was still undetermined.

It was not until after the cultures had been kept in the laboratory for four months that any spores were produced. In the fourth month the aerial mycelium produced smooth blunt-ended spindles with four or five cross-walls. At this stage of development, to the naked eye the mycelium resembled that of *Microsporon lanosum*, but the spindles were too small, had too few compartments, and were too blunt-ended to be typical for that species.

Experiments were then set up to determine whether or not hyphal fusions would take place between the mycelium derived from patient F and a mycelium from a culture of *M. lanosum* that had been definitely identified. After a few days, an abundance of fusions could be observed between the two mycelia, so that the unknown fungus was taken to be *M. lanosum*.

The determination of the mycelium from patient F as being *Microsporon lanosum* was substantiated by changes in the stock culture during the next month. The yellow color of the mycelium was gradually lost. The old

spindles fell away and were replaced by new ones. The new spindles were larger than the old ones, their tips were attenuated, their outer walls were ornamented with nodules; internally they were divided by 8-9 septa and, in general, their appearance was that of the spindles of *Microsporon lanosum*.

Thus it was not till after a lapse of six months that the fungus from patient F was satisfactorily identified by its spores, while by hyphal-fusion experiments it could have been identified a week or two after it was isolated.

It may be added that the mycelium of patient F was paired with three different mycelia of *Microsporon audouini*, with two different mycelia of *M. lanosum*, and with one mycelium of *Trichophyton gypseum* and that hyphal fusions were formed only between the mycelium from patient F and the two mycelia of *M. lanosum*.

(2) Patient H, a farm laborer, aged 19, suffering from sycosis parasitaria, showed a deep suppurating encrusted lesion two or three inches in diameter on the neck in the beard area.

Hairs were removed from the lesion and soaked in 8% caustic potash. When the hair tissue began to dissolve, a mycelium was distinctly visible both inside and outside the cuticle. The hyphae were composed of short segments which could easily be separated and they gave the appearance of a string of beads, thus suggesting that the mycelium was that of a *Trichophyton*.

A number of hairs were removed from the lesion and planted in Sabouraud's medium, and a mycelium developed from one of the hairs. The mycelium increased in size, but produced no spores even after the culture had been growing for several weeks.

In the meantime, the lesion responded readily to treatment and the patient was discharged from the hospital, but the fungus which caused the disease was still undetermined.

Experiments were then set up to find out whether or not the mycelium derived from patient H would form hyphal fusions with a mycelium from a stock culture of *Trichophyton gypseum*. Although the paired mycelia were kept growing in van-Tieghem cells for several weeks, no fusions whatsoever could be found between the unknown mycelium and the mycelium of *Trichophyton gypseum*. The negative results of these hyphal-fusion experiments led the authors to believe—contrary to their expectation— that the unknown fungus was not *Trichophyton gypseum*.

Lack of type cultures of other ectothrix *Trichophyta* prevented the authors from making further hyphal-fusion experiments, but a month later the mycelium took on a rose color and accorded very well with Sartory's description of *T. radiolatum*, Sab. (6).

According to Grigoraki (4) and others, all dermatophytes go through progressive stages of degeneration to sterility in which state it is impossible to distinguish species from one another. It remains to be shown whether or not these fungi in their sterile condition can be determined by hyphal-fusion experiments.

Finally, it is suggested that the hyphal-fusion method described in this paper

may find an application in the determination of species of fungi not only in the dermatophytes but in other Fungi Imperfecti, in the Ascomycetes, and in the Basidiomycetes.

Acknowledgment

The authors desire to acknowledge with their best thanks the courtesy of Professor William Boyd who permitted them to make use of the Pathological Laboratory of the University of Manitoba and its equipment for part of the work.

References

1. ADAMSON, H. G. Brit. Journ. Dermat. 7: 201-237. 1895.
2. BULLER, A. H. R. Researches on fungi, 3: 413-415. 1924.
3. BULLER, A. H. R. Researches on fungi, 4: 152-184. 1931.
4. GRIGORAKI, L. Ann. des Sci. Nat. 7: 165-425. 1925.
5. HENRICI, A. T. Molds, Yeasts and Actinomycetes, Ch. VI. Wiley. 1930.
6. SARTORY, A. Champignons parasites de l'homme et des animaux, 7: 405-544. 1922.
7. VIGNE, P. Marseille Medicale, 63. 1926.
8. WEIDMAN, F. D. Arch. of Derm. and Syphilology, 19: 867-877. 1929.

EXPLANATION OF PLATES I - III

PLATE I

Microsporon audouini

FIG. 1. Patient C with circular patch on scalp, within which the hairs are infected with *Microsporon audouini.*

FIG. 2. An infected hair which has been in Sabouraud's medium one week. Hyphae have grown out from the spore-sheath into the medium. Magnification, 25.

FIG. 3. *M. audouini* grown for two months on Sabouraud's medium. Natural size.

FIG. 4. Terminal chlamydospores. Magnification, 450.

FIG. 5. Mycelium from patient A showing fusions *f* between the hyphae. Mycelium grown in a hanging drop of Sabouraud's medium, ten days after inoculation. Magnification, 250.

FIG. 6. Hyphal fusions between two mycelia of *M. audouini.* The two hyphae running completely across the field from right to left are from a mycelium derived from patient A, and the four hyphae running from above downwards are from a mycelium derived from patient B. The two mycelia were paired in a hanging drop of Sabouraud's medium one month before the photograph was taken. A fusion between a hypha of the mycelium from patient A and a hypha of the mycelium from patient B is shown at f^1. Magnification, 250.

PLATE II

Microsporon lanosum

FIG. 1. Patient D, infected with *Microsporon lanosum.* The fungus has caused lesions on the eyebrow, glabrous skin, and scalp.

FIG. 2. Infected epidermis (upper left) from patient D, planted in a hanging drop of Sabouraud's medium. Hyphae of *M. lanosum* have grown out into the medium and have given rise to spindles. Magnification, 70.

FIG. 3. *M. lanosum* grown on Sabouraud's medium for five months. There is an abundance of aerial mycelium. Natural size.

FIG. 4. Spindles of *M. lanosum.* Magnification, 450.

FIG. 5. Three hyphal fusions *f f f* in the mycelium of *M. lanosum* obtained from patient D. The mycelium had been growing for one month in a hanging drop of Sabouraud's medium. Magnification, 250.

FIG. 6. Hyphal fusions between two mycelia of *M. lanosum.* The photomicrograph shows parts of two mycelia, one derived from patient D and the other from patient E, which were paired in a hanging drop of Sabouraud's medium. The arrow at *f* points to a fusion between a hypha of a mycelium derived from patient D and another hypha of a mycelium derived from patient E. Magnification, 250.

PLATE III

Trichophyton gypseum

FIG. 1. Patient G, suffering from sycosis parasitaria (tinea barbae) caused by an infection of *Trichophyton gypseum.* The lesions are in the beard areas, particularly on the upper lip.

FIG. 2. A, a diagrammatic drawing of an infected hair pulled from the beard of patient G, showing the fungal hyphae within the hair and within the follicular sheath: *b*, bulb of hair; *h*, fungal hyphae; *l*, level of skin; *s*, follicular sheath. *B*, a diagrammatic drawing of a fragment of a mycelium of *Trichophyton gypseum* growing on Sabouraud's medium *m*, to show spindles *sp* borne on an aerial hypha. Magnification, 600.

FIG. 3. *T. gypseum* grown on Sabouraud's medium for four months. Natural size.

FIG. 4. Aleuriospores formed on the aerial mycelium of *T. gypseum* growing on Sabouraud's medium in a van-Tieghem cell. Magnification, 250.

FIG. 5. Three hyphal fusions *f f f* in a mycelium of *T. gypseum* obtained from patient G. The mycelium had been growing one month in a hanging drop of Sabouraud's medium. Magnification, 250.

Fig. 6. Hyphal fusions between two mycelia of *T. gypseum.* A mycelium from patient G (of Winnipeg) and a mycelium from patient H (of New York) were paired in a hanging drop of Sabouraud's medium. The wide hypha is from one patient and the three other narrow hyphae are from the other patient. The mycelia had been growing for 13 days: each of the two lines f^1 points to a fusion between a hypha of a mycelium derived from patient G and another hypha of a mycelium derived from patient H. Magnification, 350.

PREDICTING THE VALUE OF A CROSS FROM AN F_2 ANALYSIS[1]

By J. B. Harrington[2]

Abstract

The results of extensive breeding work with the cross Marquillo x Marquis were compared with both the original expectation and the expectation calculated from a study of random F_2 populations. The cross was made for the purpose of combining the rust resistance of Marquillo with the many desirable qualities of Marquis. An F_2 population of nearly 40,000 plants was grown in order that there would be a good chance of achieving the desired combination. After five years of breeding effort (nursery, greenhouse and laboratory tests) only six lines remained and none of these were entirely satisfactory. Analysis of random F_2 populations for various important agronomic characters including stem rust reaction indicated that about seven good lines could be expected from 40,000 F_2 plants, providing genetic linkage did not interfere. As this analysis could not include baking quality, a further reduction in the number of selections could be predicted. Results on the best 27 lines from the breeding project showed that baking quality was a difficult character in this cross. The line results also indicated that genetic linkage might be concerned with respect to factors governing rust reaction, seed appearance and crumb color. It was concluded that the F_2 analysis gave a reasonably accurate prediction of the doubtful value of the cross, although it had distinct limitations with respect to characters like baking quality which could not be studied in F_2.

Usually, when a cross is made between carefully chosen parents for a definite breeding purpose, promising plants are selected in F_2 and in several succeeding generations and the cross is carried on until one or more superior new lines have been obtained or until the prospect of obtaining such lines has become very small. However, apparently desirable crosses do not always yield satisfactory results. Not frequently crosses between varieties having well-known desirable characters and no particularly unfavorable characters yield a large preponderance of progeny which could not be considered desirable. Often after a great deal of effort has been put into an attempt to attain a given combination of characters, it is found that the combination has not been fully achieved.

It appears that neither the characters of varieties nor the intensity of the expression of those characters are especially accurate indications of the results to be expected from crossing. In the writer's rust resistance breeding program which has been in progress since 1925 striking examples of good and poor crosses are available. In one case H-44-24*, a new highly rust resistant desirable bread wheat that does not thresh easily, was crossed with Double Cross†, a moderately rust resistant desirable bread wheat, and the F_1 was

[1] *Manuscript received December 2, 1931.*
Contribution from the laboratories of the University of Saskatchewan, Canada, with financial assistance from the National Research Council of Canada. This study forms a part of a co-operative attack on the problem of cereal rust in Canada, carried on jointly by the National Research Council, the Federal Department of Agriculture and the Universities of Manitoba, Saskatchewan and Alberta. The results were reported in full at the meeting of the Associate Committee on Field Crop Diseases at Winnipeg on April 9, 1931.

[2] *Professor of Field Husbandry, University of Saskatchewan.*

* *Produced by Mr. E. S. McFadden, Webster, South Dakota, from the cross Marquis x Yaroslav emmer.*

† *Produced by Dr. H. K. Hayes, University of Minnesota, from the cross (Marquis x Kanred) x (Iumillo x Marquis).*

crossed with Marquis. With comparatively little work 22 very promising resistant lines have been isolated. In contrast to this, the back cross of Marquillo with Marquis has, after six years of effort commencing with a very large F_2 population, resulted in six promising lines none of which is as promising as several of the 22 lines mentioned above.

In order to ascertain the probable economic value of a cross as soon as possible after parent varieties have been selected and the cross made, the writer suggests that a comprehensive study of a random F_2 population be made. This could be done the same season as the main F_2 population is grown or in the previous season, the main sowing being delayed a year. During the past six years a clear-cut illustration of the value of such a procedure has been obtained in the cross Marquillo × Marquis.

Procedure

A cross was made between Marquis and Marquillo in 1925. Marquis and Marquillo are both common bread wheats of normal vigor and fertility with tip awned, fusiform, mid-dense spikes and smooth white glumes. Marquis arose from a cross between Hard Red Calcutta, an Indian wheat, and Red Fife, which probably came originally from Russia. Marquillo (3) resulted from a cross between Marquis and Iumillo, a durum wheat of good yield, fair quality and extremely high rust resistance. Table I gives a comparison of Marquis and Marquillo for various agronomic characters of importance.

TABLE I

COMPARISON OF MARQUIS AND MARQUILLO FOR IMPORTANT AGRONOMIC CHARACTERS AS OBTAINED FROM PLOT AND LABORATORY TESTS COVERING SIX YEARS

Character	Marquis	Marquillo	Character	Marquis	Marquillo
Height in inches	42	*37*	Grain yield in bushels per acre	38 5	41 6
Days seeding to ripe	111	109	Grains weight per bushel	65 3	64 3
Straw strength in per cent	88	94	Seed appearance rating	100	*80*
Non-shattering in per cent	97	98	1000 kernel weight in gm.	30 6	33.0
Resistance to stem rust	*poor*	good	Milling quality score	95.8	95.1
Resistance to leaf rust	good	good	Total baking score	97	96
Resistance to bunt	fair	fair	Crumb color score	9.0	7.8
Resistance to root rot	fair	fair			

In 1926 and again in 1930 random F_2 populations of the cross Marquillo × Marquis were studied. In 1926 plant height, earliness of maturity and rust resistance were studied. During the succeeding five years, this cross was handled as an extensive breeding investigation involving nursery, greenhouse and laboratory tests and a total F_2 population of 36,800 plants was reduced to six promising lines. Owing to the difficulty of obtaining satisfactory combinations of characters in this cross, and owing to the disappointing fact that no line was obtained which fulfilled the original expectation of a combination of

Marquillo rust resistance with the milling and baking quality of Marquis, a special study of a population of several hundred F_2 plants was made in 1930. This was made possible through the keeping of a substantial reserve of F_2 seed from 1926.

In this study an analysis was made for the characters plant height, grain yield, seed appearance and rust reaction. Interrelationships of these characters were also investigated. These results, together with those of the 1926 studies, formed excellent material with which to compare the data obtained during 1929 and 1930 on the best 27 lines.

F_2 Results

Rust Reaction

A study was made of the reaction of 781 Marquillo × Marquis F_3 families in the seedling stage in the greenhouse at an average temperature of 69.6° F. to physiologic form 21 of *Puccinia graminis tritici*. Form 21 was used in the greenhouse tests because of its prominence in Western Canada and because Marquis and Marquillo react quite differently to it, Marquis being susceptible and Marquillo resistant. Only eleven, or almost exactly one sixty-fourth of the families were resistant, indicating the operation of three main genetic factors. The results are shown in Table II.

TABLE II

DISTRIBUTION OF 781 F_2 PLANTS* OF THE CROSS MARQUILLO × MARQUIS, ACCORDING TO THE REACTION OF THEIR F_3 SEEDLING PROGENY TO FORM 21 IN THE GREENHOUSE AT A TEMPERATURE OF APPROXIMATELY 70° F.

Material	Distribution of F_2 plants according to the classes determined by the reaction of F_3 seedlings					Number of F_3 families
	R**	HR	H & I	HS	S	
Marquis					15	
Marquillo	13					
F_2	11	29	91	261	389	781

* *This was not a complete random sample with respect to rust reaction, since approximately 50 of the most susceptible plants were discarded in the field. Even with these 50 added the fit to a three factor hypothesis remains quite good.*

***R, resistant; HR, heterozygous resistant; H, heterozygous; I, intermediate; HS, heterozygous susceptible; S, susceptible.*

Field nursery results in 1926 and 1927 indicated that three or more factors controlled the reaction to the forms of rust present. Tests from various field rust cultures showed the presence of only form 21 in the 1926 nursery, but in the epidemic year 1927 there were present forms 15, 17, 21, 29, 30 and 36. Form 21 appeared to be the most abundant of these.

Both greenhouse tests to form 21 and the field tests indicated the existence of three main genetic factors for rust reaction. The value of these results was increased by finding that there was a strong positive relationship between the

seedling and the field reaction†. This information was obtained from a study of 129 F_3 families with respect to the correlation between their seedling reactions to form 21 and their field reactions in 1927 to a mixture of forms. The coefficient of contingency was 0.69 ± 0.062.

In December 1930 several hundred F_3 families of the F_2 population grown during the preceding summer were tested to form 21 in the seedling stage. The results agreed well with the previous results in indicating three genetic factors governing the reaction.

From the breeding standpoint the significant feature of these findings was that only about 1.5% of the F_2 plants resembled the Marquillo parent variety in rust reaction.

TABLE III

DISTRIBUTION OF MARQUILLO × MARQUIS HYBRIDS FOR DAYS FROM SEEDING TO MATURITY

Material	Class centres for days from seeding to maturity							Av. no. days, seeding to ripe
	50	53	56	59	62	65	68	
Marquis		13	94	49	16	6		57.4 ± 2.33
Marquillo	6	91	61	8				54.3 ± 0.94
F_2 plants	7	55	231	62	23	10	3	56.6

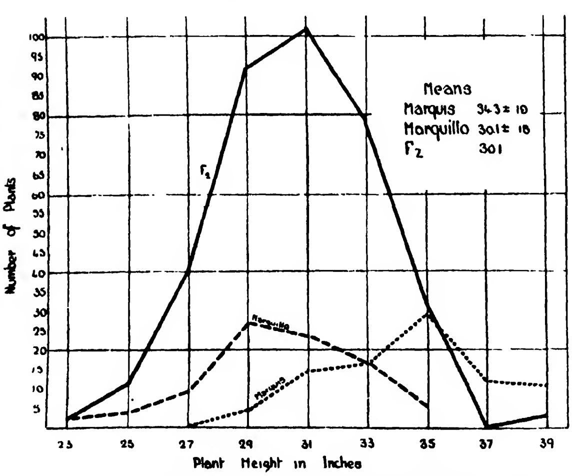


FIG. 1. *Marquillo × Marquis plant height, 1930.*

Earliness of Maturity

Marquillo is about two days earlier in maturity than Marquis, as shown in Table I. The F_2 population showed no definite indication of dominance (see Table III). In this cross an attempt to obtain segregates with the earliness of Marquillo might mean the loss of a fairly large proportion of the hybrids. Therefore, since a two day difference in time of maturity is not especially important, there would seem to be no good reason to discard any of the hybrids on this basis.

Height of Plant

Height of plant is important where short straw frequently occurs in dry seasons and the binder is the usual harvesting machine. Marquillo is several inches shorter than Marquis. The F_2 results in 1926 as well as in 1930 indicated that this character was probably dependent on several genetic factors. There was no clear-cut indication of dominance, but the F_2 in the 1930 study had

† *Previously Harrington and Smith (2) had noted this relationship.*

the same average height as Marquillo (see Table IV). Only 35 F_2 plants equalled the average height of Marquis and more than two-thirds of the F_2 plants were three inches or more shorter than Marquis. The results on height are shown graphically in Fig. 1. It would seem to be desirable to discard the shortest plants to the extent of about a third of the population. The chances remained that most of the retained hybrids would not excel Marquillo in height. On the other hand, the height of Marquillo could hardly be considered a sufficiently important disadvantage to warrant discarding much more than the plants below the main Marquis range in height.

TABLE IV

PLANT HEIGHT OF MARQUILLO x MARQUIS HYBRIDS, 1930

Material	Distribution of plants according to their height in inches									Average plant height
	23	25	27	29	31	33	35	37	39	
Marquis			1	4	15	17	29	13	11	34.8 ±0.19
Marquillo	2	4	8	26	23	17	5			30.6 ±0.18
F_2	7	11	40	90	102	78	32	1	2	30.6

Seed Characters

Seed character refers principally to milling and baking quality, shape, plumpness, color (lustre) and size. These characters are particularly important where a conservative, exacting foreign market is to be suited. Unfortunately, it is not possible to obtain milling and baking data on single plants. As for the other characters, it is possible by careful laboratory inspection to combine in a single expression the value of a line with respect to all of them. This was done in the 1930 F_2 study under the term "seed appearance." The results appear in Table V and are shown graphically in Fig. 2. It is apparent that there is a decided preponderance of the seed characters of Marquillo in the F_2. Only six of the 356 F_2 plants equalled the Marquis mean in seed appearance. It would seem necessary therefore to discard a very large proportion of the hybrids in order to retain the seed appearance of Marquis.

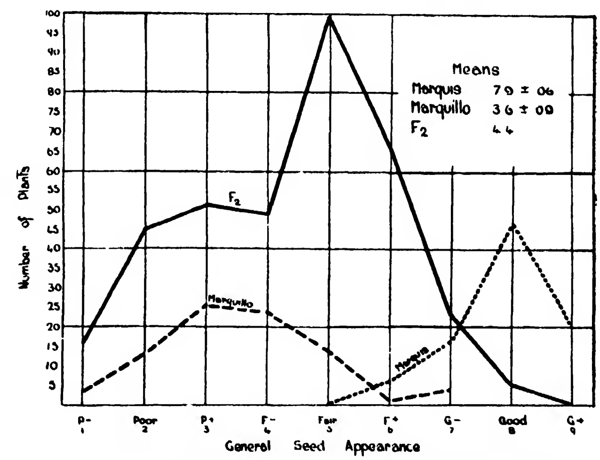


FIG. 2. *Marquillo × Marquis seed appearance, 1930.*

Grain Yield

Grain yield is generally considered one of the most important characters in a cereal crop. Unlike height of plant, earliness of maturity and other

TABLE V

SEED APPEARANCE OF MARQUILLO × MARQUIS HYBRIDS, 1930

Material	Distribution of plants according to their seed appearance taken on the basis of shape, color and plumpness									Average seed appearance
	P− 1	Poor 2	P+ 3	F− 4	Fair 5	F+ 6	G− 7	Good 8	G+ 9	
Marquis					1	6	16	47	20	7.9 ± .06
Marquillo	3	13	26	24	14	2	3			3.6 ± .09
F_2	16	45	52	49	99	66	23	5	1	4.4

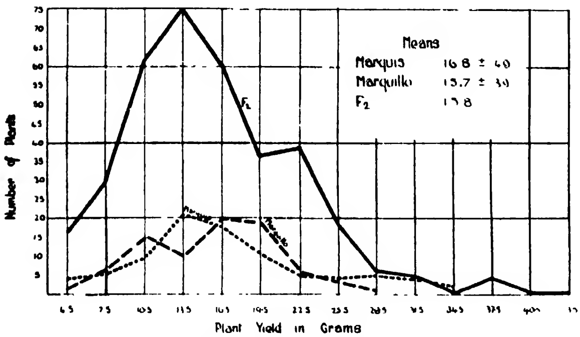


FIG. 3. *Marquillo × Marquis grain yield, 1930.*

characters discussed, grain yield is not usually obtained on hybrid lines until sufficient seed is available for a proper rod row replicated plot test. This is usually not until F_4 at the earliest and frequently not until F_5 or F_6. To obtain a reliable index of yielding ability in a given cross and especially to obtain relationships between yield and other important characters before the cross had progressed beyond the first segregating generation would be highly desirable. This may be accomplished with F_2 plants. The actual yield of any given plant tells very little respecting the inherited yielding ability of that plant, but the actual yields of all the individual plants in a population give a reliable estimate of the yielding ability of the cross as a whole.

TABLE VI

DISTRIBUTION OF MARQUILLO × MARQUIS HYBRIDS FOR GRAIN YIELD, 1930

Material	Class centres for grain yield in grams per plant							Average plant yield
	6	12	18	24	30	36	42	
Marquis	10	30	30	9	9	2		16.8 ±0.49
Marquillo	9	25	39	10	2	.		15.7 ±0.39
F_2	47	137	97	56	12	5	2	15 8

The F_2 results on grain yield are summarized in Table VI. The parent varieties did not differ significantly in yield. The apparent higher yield of Marquis is in fact opposite to the usual plot results where Marquillo generally yields higher, as shown in Table I. The F_2 curve for yield as shown in Fig. 3 closely approaches those of the parent varieties, indicating that no trouble should be anticipated with respect to that character.

Analysis of the F_2 Results for the Estimation of the Probable Success of the Cross

The F_2 results may be used in predicting the probable possibility of obtaining hybrid lines combining the desirable characters of the parent varieties as far as the characters studied are concerned. The rust reaction results showed that about one sixty-fourth of the hybrids would probably be homozygous for Marquillo resistance. The results on earliness indicated that no attempt to select for earlier maturity than that of Marquis would be advisable as the sacrifice of population would hardly be justified by the additional earliness. The results on plant height indicated the desirability of discarding the shortest one-third of the population. The seed appearance results showed that the chances of having Marquis appearance in selected segregates were small, for only one-sixtieth of the F_2 plants equalled the Marquis mean. Results on grain yield did not show the necessity of discarding any hybrids.

The foregoing analysis may be summarized numerically in order to obtain a figure representing the proportion of F_2 plants that would probably be satisfactory for the characters studied. The calculation is as follows:— $\frac{1}{64} \times \frac{2}{3} \times \frac{1}{60} = \frac{1}{5760}$. That is, one plant in every 5760, or approximately 7 plants from a population of 36,800 (the actual F_2 population) should be satisfactory for the characters studied providing the factors for each character were independently inherited. Just how much these 7 individuals would be reduced in number on the basis of milling and baking quality or any other character which could not be studied, remains unknown.

Results on the Best Hybrid Lines Arising from the Breeding Project

An extensive breeding project was commenced in 1925 with the cross Marquillo × Marquis for the purpose of combining the desirable characters of these two varieties. As seen from the description of Marquillo and Marquis in Table I, the goal would not appear to be particularly difficult to attain.

TABLE VII

REDUCTION OF THE NUMBER OF MARQUILLO × MARQUIS LINES DURING THE FIRST SEVEN GENERATIONS

Number of lines		Generation	Year	Basis of elimination
Grown	Discarded			
36,800	32,600	F_2	1926	Field rust reactions, height, earliness, vigor.
4,200	3,781	F_2	1926–7	Seedling reaction of F_3 progenies to form 21.
519	417	F_3	1927	Field rust reaction, also earliness, etc.
102	19	F_4	1928	Preliminary replicated plot test for yield, etc. milling and baking test.
83	56	F_5	1929	Replicated plot tests, root rot tests, milling and baking tests.
27	21	F_6	1930	Same as in 1929 and smut tests
6		F_7	1931	Same as in 1930

Nevertheless, in order to be reasonably sure of satisfactory results, a large F_2 population was used. During the course of six years of nursery, greenhouse and laboratory tests, this population was reduced to six good lines, none of which fulfilled the original expectations. The reduction in the number of lines from year to year is shown in Table VII.

As shown in Table VII, there were 83 lines remaining under test in 1929. Fifty-six of these were discarded in the fall on account of low yield and insufficient rust resistance. In the following summarization of results for the remaining 27 lines data from replicated rod row tests covering three years have been used. It will be seen that the practical value of lines has been considered and the theoretically desirable elimination of any hybrid not measuring up fully to the best characters of both parent varieties has not been rigidly practiced.

TABLE VIII

DISTRIBUTION OF THE 27 LINES ACCORDING TO THEIR RUST REACTION ON THE BASIS OF THE RESISTANCE OF MARQUILLO*

More R	As R	10% less R	20% less R	30% less R	40% less R	50% less R	60% less R	70% less R
1	2	2	4	5	2	5	4	1

* *Marquis was approximately 300% less resistant than Marquillo.*

Rust Reaction

Seedling tests to pure cultures of form 21 have shown the 27 best F_6 lines to be fairly rust resistant with a range extending from the resistance of Marquillo to a much lesser amount, and averaging distinctly less resistant than Marquillo. Only three of the 27 best lines were as resistant as Marquillo. The results of nursery tests in 1930 on all of the 27 lines at Indian Head, Grenfell and Carmichael, of part of them at Winnipeg, St. Paul and Fargo, and of part of them at Saskatoon in 1928 are summarized in Table VIII.

TABLE IX

DISTRIBUTION OF THE 27 LINES FOR TIME FROM SEEDING TO MATURITY IN PERCENTAGE OF MARQUIS (AVERAGE 1929 AND 1930 RESULTS)

Material	98	99	100
Marquis			×
Marquillo	×		
Hybrid lines	9	15	3

Earliness of Maturity

No selection was made upon the basis of earliness of maturity as both parent varieties mature moderately early, consequently, the line results would be expected to agree fairly well with those from the F_2 study. Table IX, giving the summarized data, shows this to be the case.

Height of Plant

In the first segregating generation each field resistant plant was measured for height when harvested and only those at least 95% as tall as the nearest Marquis check plots were kept. Approximately one-third of the population

was discarded upon this basis. However, the F_2 analysis indicated that very few plants would be likely to yield lines resembling Marquis in height. The actual line results show, as summarized in Table X, that none of the lines equalled Marquis in height. But the general average for the lines was higher than that of the random F_2 population, as was expected.

TABLE X

DISTRIBUTION OF THE 27 LINES FOR HEIGHT OF PLANT IN PERCENTAGE OF MARQUIS (AVERAGE 1929 AND 1930)

	85.5	87.5	89.5	91.5	93.5	95.5	97.5	99.5
Marquis								×
Marquillo					×			
Hybrid lines	1		3	3	9	9	2	

Seed Characters

In addition to a combined expression representing for each line its seed character as explained in connection with the F_2, separate notes were taken on the lines for bushel weight, seed plumpness and seed color. The summarized results are shown in Tables XI to XIV. In bushel weight and seed plumpness the lines averaged closer to Marquillo than to Marquis, but a fair proportion of hybrids in each case were about as good as Marquis. For seed color (lustre) the poor appearance of Marquillo had a very dominating influence and only one of the 27 lines was like Marquis.

TABLE XI

DISTRIBUTION OF THE 27 LINES FOR WEIGHT PER MEASURED BUSHEL IN PERCENTAGE OF MARQUIS (AVERAGE 1929 AND 1930 RESULTS FROM REPLICATED ROD ROW PLOTS)

	95	96	97	98	99	100	101
Marquis						×	
Marquillo			×				
Hybrid lines	2	4	6	9	4	1	1

TABLE XII

DISTRIBUTION OF THE 27 LINES FOR SEED PLUMPNESS IN PERCENTAGE OF MARQUIS (AVERAGE 1929 AND 1930 RESULTS FROM REPLICATED PLOT TESTS)

	Class centres for seed plumpness						
	88	91	94	97	100	103	106
Marquis					×		
Marquillo				×			
Hybrid lines	1	3	5	9	6	2	1

The combination character "seed appearance" expresses best the apparent value of a variety as seen from visual examination of a handful of seed. The data on this character (see Table XIV) resemble closely those of the F_2 in

Table V, excepting that the line data show an absence of lines poorer than Marquillo. This difference is due to the fact that each year lines with very poor seed appearance were discarded.

TABLE XIII

DISTRIBUTION OF THE 27 LINES FOR SEED COLOR (LUSTRE) IN PERCENTAGE OF MARQUIS (RESULTS FROM 1930 QUADRUPLICATE PLOT TESTS)

	80	84	88	92	96	100
Marquis						×
Marquillo		×				
Hybrid lines		5	8	8	5	1

TABLE XIV

DISTRIBUTION OF THE 27 LINES FOR GENERAL SEED APPEARANCE IN PERCENTAGE OF MARQUIS (RESULTS FROM THE QUADRUPLICATE ROD ROW PLOT TEST IN 1930)

	80	84	88	92	96	100
Marquis						×
Marquillo	×					
Hybrid lines	2	4	10	9	1	1

TABLE XV

DISTRIBUTION OF THE 27 LINES FOR GRAIN YIELD IN PERCENTAGE OF MARQUIS (RESULTS FROM THE QUADRUPLICATE ROD ROW PLOT TESTS IN 1929 AND 1930)

	Class centres for mean yields of lines					
	81	86	91	96	101	106
Marquis					×	
Marquillo						×
Hybrid lines	1	2	7	10	5	2

Grain Yield

The line results on grain yield were very surprising, for, as shown in Table XV, the average yield of the lines is considerably below that of the parent varieties, whereas in the F_2 results the mean was intermediate with respect to the parent variety means. The expectation was that nearly all the lines would be high in yield, since both parent varieties are high and since the general tendency throughout the selection in the main segregating generations (F_2, F_3 and F_4) would be to eliminate lines that appeared to be distinctly inferior in yielding capacity. Genetic linkage seems to be involved.

Milling and Baking Quality

Both Marquillo and Marquis mill well and in the eyes of Minnesota millers, they are almost equally desirable in baking quality. The chief difference between these varieties is that flour from Marquillo has much more carotin pigment than Marquis flour. However, the millers find that the slightly yellowish color is taken out as far as is necessary by bleaching. The distribution of the hybrid lines with respect to crumb color is given in Table XVI. The fact that most of the lines showed distinct inferiority to both parent varieties in 1930 is inexplicable.

Marquis and Marquillo were closely alike in computed baking score where bleached flour is used. As most millers regularly bleach flour, the results on the lines are presented in Table XVII on that basis. The results were even more striking and inexplicable than in the case of crumb color. In 1929 no

TABLE XVI

DISTRIBUTION OF THE 27 LINES FOR CRUMB COLOR IN PERCENTAGE OF MARQUIS AS OBTAINED FROM AVERAGING THE RESULTS FROM THE "BROMATE" AND THE "BLEND-BROMATE" BAKING METHODS USED ON BLEACHED FLOUR

	75-78	79-82	83-86	87-90	91-94	95-98	99-102	103-106	107-110
Marquis							1928-30		
Marquillo			1930		1928	1929			
Hybrid lines 1928*									4
Hybrid lines 1929			4	12	3	5	2		
Hybrid lines 1930*	2	1	3	2					

* *Many of the lines were not tested.*

line averaged as high a score as the parent varieties and nearly a third of them were distinctly low, whereas in 1928 and in 1930 all the lines tested gave good results.

TABLE XVII

DISTRIBUTION OF THE 27 LINES FOR COMPUTED BAKING SCORE IN PERCENTAGE OF MARQUIS AS OBTAINED FROM AVERAGING THE RESULTS FROM THE "BROMATE" AND THE "BLEND-BROMATE" BAKING METHODS USED ON BLEACHED FLOUR

	79-82	83-86	87-90	91-94	95-98	99-102	103-106	107-110
Marquis						×		
Marquillo					1928	{1929 1930		
Hybrid lines 1928*						2	1	1
Hybrid lines 1929	2	5	11	6	2			
Hybrid lines 1930					3	4		1

* *Many of the 27 lines were not tested.*

Reaction to Root-rotting Organisms

The Marquillo × Marquis lines and the parent varieties were tested for their reaction to some common root-rotting organisms in a special disease garden and in the greenhouse in 1929, and in the disease garden and at Grenfell, Carmichael and Humboldt in 1930. The 1929 disease garden test was unsatisfactory due to droughty conditions. The greenhouse test was made at the seedling stage to virulent strains of *Helminthosporium sativum*, *Fusarium culmorum* and *Ophiobolus graminis*. The results were not particularly striking. The parent varieties appeared to be about equally subject to seedling infection excepting that Marquillo seemed slightly more susceptible to *F. culmorum* than Marquis. None of the lines showed higher infection than the parent varieties.

The 1930 tests at the three points outside Saskatoon gave unsatisfactory results, due to the dry season. The results from the disease garden are summarized in Tables XVIII and XIX. No significant difference in reaction was found with respect to the two varieties and their hybrids either for lesioning of the crown or for number of blighted plants.

TABLE XVIII

Distribution of Marquillo x Marquis hybrid lines and Marquillo with respect to their difference from the nearest Marquis checks in average index of lesioning

Cultures	Class centres for differences from Marquis checks in index of lesioning $\left(\begin{matrix} - = \text{less lesioning} \\ + = \text{more} \end{matrix}\right)$							
	−12	−9	−6	−3	0	+3	+6	+9
Marquillo	1			3	1	1		1
Hybrid lines	1	3	8	6	14	9	1	

TABLE XIX

Distribution of Marquillo x Marquis hybrid lines and the parent varieties with respect to their difference from the nearest Marquis checks in total number of blighted plants

Cultures	Class centres for differences from Marquis checks in number of blighted plants						
	−21	−14	−7	0	+7	+14	+21
Marquillo			2		3	1	1
Hybrid lines	3	7	5	9	8	7	3

As Marquis does not suffer greatly from root rot under ordinary field conditions in Western Canada, the hybrid lines may be considered reasonably satisfactory as far as the results of these tests are concerned.

Reaction to Covered Smut

The parent varieties and hybrid lines were tested in duplicate plots for bunt reaction in 1930. The average percentages of plants not infected were as follows:

Marquis, 33 ± 1.7. Marquillo, 32 ± 1.7. Hybrid lines, 35.

Marquis and Marquillo were not significantly different for bunt reaction and the hybrid lines averaged about the same as the parent varieties. Marquis under ordinary field conditions is moderately susceptible to bunt.

Relationships Between Characters

The interrelationships of plant height, seed appearance and grain yield were studied in the F_2 data. The following correlation coefficients were obtained:

Grain yield and plant height.................. 0.57 ± 0.024
Grain yield and seed appearance............... 0.25 ± 0.034
Plant height and seed appearance.............. 0.47 ± 0.028

These values show distinct relationships, but as they could easily be largely the result of environmental influences, similar interrelationships were worked

out for the data on the Marquis parent plants of which 90 were studied. The coefficients are as follows:

Grain yield and plant height	0.78 ± 0.028
Grain yield and seed appearance	0.50 ± 0.053
Plant height and seed appearance	0.59 ± 0.046

The Marquis results show relationships fully as high as those of the F_2, consequently it cannot be said that the latter indicate genetic linkage.

In addition the relationship between the yields of F_2 plants and the seedling reaction of their F_3 progenies to stem rust form 21 was obtained; r was 0.08 ± 0.050. Similarly for plant height and rust reaction the value of r was 0.10 ± 0.047.

Interrelationships were then worked out for the results on the 27 best lines. The correlations were as follows:

Grain yield and computed baking score	0.2 ± 0.13
Grain yield and rust resistance*	0.1 ± 0.13
Grain yield and seed plumpness	0.4 ± 0.11
Grain yield and crumb color	0.2 ± 0.13
Grain yield and seed appearance	0.1 ± 0.13
Crumb color and seed appearance	0.6 ± 0.09
Rust resistance and seed appearance*	0.3 ± 0.12
Crumb color and rust resistance*	0.3 ± 0.12
Plant height and rust resistance	0.1 ± 0.13
Plant height and computed baking score	0.2 ± 0.13

There were only two relationships that appeared to be significant. One concerns seed plumpness and grain yield and probably has no genetical significance since such a relationship would be expected as a result of environmental effects. The other relationship is between crumb color and seed appearance and may have some genetical importance. It is apparent that the study of character interrelationships does not explain the line results shown in Tables XIV, XV and XVI.

Discussion

Every year scores of new crosses are made in the small grains at the various experimental stations scattered across the country and many of these crosses never result in worthwhile new varieties. It may be assumed that most of these crosses are made after careful thought had been given to the choice of parent varieties. The conclusion is then obvious that apparently good combinations of varieties do not always prove satisfactory. This being the case, the sooner critical tests can be made of the probable practical value of the crosses, the greater will be the amount of time and energy available for

* *The usual physical relationships between rust reaction and other characters did not affect this correlation as the data on yield, seed appearance and crumb color were secured at Saskatoon as summarized in Tables XIV, XV and XVI, whereas the rust data were averaged from 1930 tests in a number of localities.*

use upon those that are really promising. The F_2, or first segregating generation, offers the earliest opportunity for such critical tests. The present study illustrates the use and value of an F_2 study for this purpose.

The desirability of a cross as far as disease resistance is concerned, depends not only upon the degree of resistance of the resistant parent variety but also upon the frequency with which desirable resistance occurs in the hybrids and upon relationships between resistance and undesired characters. Marquillo has sufficient stem rust resistance to insure a farmer a reasonable degree of security against heavy loss from rust. The substitution of Marquillo for the older rust susceptible varieties, such as Marquis, has been going on for several years in Minnesota and testifies to the practical value of the new variety. However, when an attempt was made to improve upon Marquillo by backcrossing it with Marquis, one of the greatest obstacles was the paucity of resistant progeny.

The F_2 results showed that only about one sixty-fourth of the hybrid plants possessed resistance as high as that of Marquillo. All of the rest of the plants were more or less susceptible. Most of these would have yielded some resistant plants upon segregation of their progeny but to await these further generations for the selection of more resistant plants would have taken valuable time and increased the cost and complexity of the work. Consequently it was considered that approximately 98.5% of the F_2 population was of little value and warranted discarding. This was done but the loss was heavy since, for the sake of one character, 98.5% of the work of crossing and of growing, examining and testing the F_1 and the F_2 was lost. The evidence furnished later by the line results in F_4, F_5 and F_6 demonstrated that such an initial loss is a severe handicap for it reduces the population so greatly that insufficient material is likely to be left to furnish the desirable combinations of characters for which the cross was made. This line evidence indicated that a very heavy elimination of progeny for a single unfavorable character at the outset of a cross would probably warrant dropping the cross without going further, especially if a number of other important characters were still to be considered.

For yield and earliness, both parent varieties were satisfactory and the F_2 distributions, as expected, resembled the distributions for the parent varieties fairly closely. There appeared to be no reason for discarding hybrids on the basis of these characters. The F_6 line results showed that the F_2 analysis correctly predicted no difficulty as to earliness.

However, the results on the best 27 lines remaining after five years of breeding showed 20 of these lines to be inferior to both parent varieties in yielding ability. This was not expected, as both parent varieties yielded well and Marquillo, the rust resistant variety, excelled Marquis, the susceptible variety, in this respect. The line results suggest genetic linkage between factors for rust susceptibility and factors for yielding capacity. This was not proved however. The lines varied from one that was more resistant than Marquillo to one that was 70% less resistant than that variety, yet no correlation indicating genetic linkage was found between rust reaction and yield. Furthermore no indication of genetic relationship was discovered

between grain yield and rust reaction in the F_2 study. This was as expected since the inheritance of rust reaction is complex and that of yielding ability has been found in other crosses to be complex as shown by the studies of Waldron (4), Bridgford and Hayes (1) and others. It may be concluded that the F_2 results on yield were somewhat misleading.

For height of plant, the F_2 distribution was practically identical with that of Marquillo. This indicated that the hybrid lines would on the whole resemble Marquillo in height. Discarding the shortest hybrid plants to the extent of 35% would remove most of any short plants resulting from possible transgressive segregation. This would result in the 27 selected lines averaging slightly taller than Marquillo. The results in Table X accord with this supposition. Therefore, the F_2 results on plant height correctly intimated what was to be expected from the selected lines.

Seed characters are extremely important, particularly grain plumpness, color and quality. F_2 results fail to predict milling and baking quality as no reliable tests have yet been devised for detecting such qualities in the small amount of seed obtainable from a single plant. In the cross Marquillo × Marquis, no great difficulty as to quality was anticipated owing to the desirability of both parent varieties in this regard. It is true that Marquillo is less desirable than Marquis in flour color and, on that account, in general baking score, but the writer understands* that United States millers and bakers who are accustomed to handling Marquillo, like it about as well as Marquis. In protein content, water absorption and loaf volume, Marquillo flour ranks almost equal to that of Marquis. The line results were a great surprise for they showed the lines to have poorer quality than Marquis in both 1929 and 1930 although better quality in 1928. The selected lines averaged only slightly higher in quality than Marquillo. If a milling and baking test on the F_2 plants had been possible, the latter results on the lines might have been predicted. This would have been a further reason for considering the cross undesirable to continue.

F_2 results may be used to estimate the possibilities of the cross as far as morphological seed characters are concerned. A general note was taken on the seed of each plant for general appearance which included plumpness, color, shape and size. Only 6 of the 356 F_2 plants equalled the Marquis mean in seed appearance Here again, as with rust reaction, it would seem necessary to discard over 98% of all the hybrids if only those equalling Marquis in seed character were to be saved. If this test for seed appearance had been made in 1926 instead of in 1930, it is probable that the results added to those obtained on rust and plant height would have been considered sufficient to justify discarding the cross without carrying it further. However, a critical study of F_2 for seed appearance was not made at the beginning of the investigation, but the lines that were poor in seed appearance were discarded during the first four generations. Of the best 27 lines, only one equalled Marquis in

* *From a statement by Dr. R. K. Larmour regarding the high regard for Marquillo expressed by Minneapolis millers at a recent conference of cereal chemists.*

seed appearance. The result agrees very well with the F_2 analysis and reveals again the value that would have existed in a study of an experimental F_2 before the breeding project was carried forward in a large way.

Summing up, the results of the present study indicate that a preliminary experimental F_2 population of several hundred plants should be analyzed for all important characters at or before the commencement of extensive work on a cross. In the Marquillo × Marquis cross the F_2 was quite satisfactorily used for a thorough individual plant study of stem rust reaction, plant height, earliness of maturity, yield of grain and various seed characters. Other characters could also have been used, including tillering, protein content (where the amount of seed per plant is plentiful) resistance to frost, weathering and sprouting, reaction to leaf rust, root rots, smuts, hot winds, etc. In the present study, part of the F_2 analysis was made on a specially grown random population several years after the commencement of the cross. This later F_2 study was made primarily to explain why none of the best F_6 lines combined all of the favorable characters of the two parent varieties as was desired. The analysis of the two F_2 populations and the linking up of the results with those from the best F_6 lines has shown definitely how advantageous a complete F_2 study would have been in the beginning.

As was stated earlier in this paper, the Marquillo × Marquis cross, while it has produced several meritorious lines, each of which appears to excel Marquillo distinctly in general value, did not fulfil the writer's original expectations and has been much less worthwhile than other crosses, among which may be mentioned the (H-44-24 × Double Cross) F_1 × Marquis cross. Undoubtedly among the crosses made each year in Canada there are many corresponding in comparative value to the two crosses above mentioned. It would therefore seem highly desirable to make an F_2 analysis as outlined here and then discard the cross immediately if the chances for success without great effort and cost are not reasonably large. Where such analysis convinces the investigator that the cross is worth continuing, it is very probable that the data accumulated in the F_2 study will be a valuable aid in the annual selection of desirable plants. As a matter of fact, in most crosses, an analysis of some characters is usually made in F_2 for the purpose of assisting the selection work.

While the advantages of a preliminary F_2 analysis are large, it should be remembered that an F_2 distribution may sometimes be misleading. Furthermore, genetic linkage may be present as a distinct obstacle to the accomplishment of the breeding purpose. Ascertaining the relations between various characters of the F_2 is therefore advisable.

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References

1. Bridgford, R. O. and Hayes, H. K. Correlation of factors affecting yield in hard red spring wheat. J. Am. Soc. Agron. 23 : 106-117. 1931.
2. Harrington, J. B. and Smith, W. K. The relation of wheat plants at two stages of growth to stem rust. Sci. Agr. 8 : 712-725. 1928.
3. Hayes, H. K., Stakman, E. C. and Aamodt, O. S. Inheritance in wheat of resistance to black stem rust. Phytopathology, 15 : 371-387. 1925.
4. Waldron, L. R. A partial analysis of yield of certain common and durum wheats. J. Am. Soc. Agron. 21 : 295-309. 1929.

A COMPARISON OF GLUTENIN AND GLIADIN PREPARED FROM ONE FLOUR BY VARIOUS METHODS[1]

By R. K. Larmour[2] and H. R. Sallans[3]

Abstract

Gliadin and glutenin were prepared from gluten of hard red spring wheat flour by five different methods and analyzed by the Van Slyke procedure. The gliadin preparations gave very similar results, indicating that differences in manipulation have little effect on this protein. The glutenin preparations showed very great differences in nitrogen distribution, the greatest being in ammonia nitrogen and in basic nitrogen. There is considerable evidence that these two nitrogen fractions tend to be inversely proportional. This is particularly applicable to preparations made by methods involving use of alkaline solutions. Glutenin isolated by use of Blish and Sandstedt's acetic acid method, using 0.007 *N* acid appeared to be purer than the other preparations and contained the highest percentage of ammonia nitrogen. This protein, when dissolved in 0.025 *N* sodium hydroxide solution and allowed to stand for one week, lost 4.8% of its nitrogen in the form of ammonia, but the basic fraction was unchanged. It seems likely that any method for isolating glutenin involving use of alkaline solutions would involve loss of nitrogen in the course of the preparation and the nitrogen distribution obtained by the Van Slyke analysis would therefore be in error. The use of very dilute acetic acid is recommended but exposure to even dilute solutions of the strong alkalies should be rigorously avoided in the preparation of glutenin.

Since the demonstration of their existence, the gluten proteins, gliadin and glutenin, have appealed to investigators as the constituents of flour most likely to influence the baking strength. This is a logical conclusion, as from the nature of crude gluten one would expect it to be responsible for the elasticity and gas-retaining properties of the dough. As these two proteins comprise approximately 90% of the total flour protein, there have been numerous attempts to discover a direct relationship between protein content of wheat and flour with the strength as determined by the baking test. A high degree of correlation between these variables would warrant the use of protein, as calculated from the Kjeldhal nitrogen, in estimating the commercial worth of any particular sample. The investigations made by Zinn (21), Mangels (19), and Hayes, Immer and Bailey (9), show that a relationship undoubtedly exists, but that the quantity of protein alone is not an adequate measure of strength. The variability not accountable to variation in amount of protein has been generally attributed to another factor, namely, the quality of the protein. Although Larmour (17, 18) has shown that by use of the Werner bromate formula for experimental baking much of this unaccountable variation is eliminated, there still remain differences between wheat flours that cannot be wholly related to the quantity of protein present. This variability may be

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attributable to qualitative differences or to manipulative error, principally in the baking test, or to both causes.

If qualitative differences do exist they may be attributable in part to variation in either chemical or colloidal properties of the proteins. Not a great deal of comparative work on chemical composition of these proteins has been done. Blish (1) prepared and analyzed glutenin and gliadin from both a weak and a strong flour but was unable to find any significant differences in the various nitrogen fractions as determined by the Van Slyke procedure. Cross and Swain (5) carried out a similar study using flour milled from four varieties of wheat and came to the conclusion that neither the gliadins nor the glutenins from the various flours could be chemically differentiated. Hoffman and Gortner (10) concluded "that the gliadin prepared from different varieties of wheat, including wheats which give strong and weak flours, are identical . . . . This identity apparently holds for physical as well as chemical properties." Without any very extensive work on the subject, there seems to have arisen the general belief that proteins from weak and strong flours cannot be differentiated by means of the Van Slyke distribution. However, when one compares the results of analyses reported by different investigators, very wide ranges in values of many of the nitrogen fractions are noted. Thus, in data collected from the literature by Larmour (16), variations are found as shown in Table I.

TABLE I

RANGE OF VARIATION OF NITROGEN FRACTIONS OF GLIADIN AND GLUTENIN PREPARED FROM WHEAT (*T. vulgare*) FLOUR. (TAKEN FROM TABLES III AND IV LARMOUR (1928))

Nitrogen fraction	Range of values	
	Gliadin	Glutenin
Ammonia N	24 6–26.8	13.1–16.5
Arginine N	4.6– 5.7	8.2–12.9
Total basic N	11.0–14.4	18 0–26.2
Amino N of filtrate	51.4–54.1	53.4–58.2
Non-amino N of filtrate	4.4–10.7	2.6– 9.5

The range in gliadin analyses is fairly small and one might almost be justified in attributing the differences to experimental errors. The very wide variations noted in the data for glutenin, however, can scarcely be attributed to this cause. They might be due to a combination of differences of technique of preparation and analysis. In order to get information on the error to be expected in two samples obtained by one method, two preparations of gliadin and glutenin were made by the Blish and Sandstedt (3) method. These were analyzed in duplicate and the results are shown in Table II. It is apparent that the variation between preparations is no greater than the variation in duplicate analyses. On the basis of these data it may be concluded tentatively that it is possible to replicate preparations of proteins in one laboratory. The question whether different men using the same procedure in different laboratories could make as good replication remains to be investigated.

At this point it was planned to prepare and analyze proteins from a series of flours of graded strength in order to ascertain if any chemical differences could be discovered. However, in deciding to prepare flour proteins one is at once confronted with choice of method, as there are a number of procedures,

TABLE II

VAN SLYKE NITROGEN DISTRIBUTION OBTAINED WITH DUPLICATE PREPARATIONS BY THE BLISH AND SANDSTEDT (1929) METHOD (USING 0.007 N ACETIC ACID)

Nitrogen fraction	Preparation A			Preparation B		
	1	2	Mean	1	2	Mean
Gliadin						
Ammonia N	25.80	25.85	25.82	25.83	25.62	25.72
Total humin N	0.85	0.68	0.77	0.87	0.82	0.84
Total basic N	10.72	10.33	10.53	11.22	11.07	11.14
Arginine	5.41	5.39	5.40	5.17	5.30	5.23
Cystine	0.61	0.96	0.79	0.63	0.67	0.65
Histidine	4.31	3.30	3.79	4.67	4.45	4.56
Lysine	0.39	0.70	0.55	0.75	0.15	0.70
Total filtrate N	62.98	62.84	62.81	61.91	62.05	61.98
Amino	53.59	53.48	53.53	53.86	53.98	53.92
Non-amino	9.39	9.16	9.28	8.05	8.07	8.06
Total	100.35	99.52	99.93	99.83	99.56	99.68
Glutenin						
Ammonia N	21.46	21.31	21.38	20.58	20.58	20.58
Total humin N	1.36	1.44	1.40	1.59	1.40	1.49
Total basic N	17.87	18.45	18.16	17.77	17.87	17.82
Arginine	8.47	8.39	8.43	8.52	8.69	8.60
Cystine	0.96	0.92	0.94	0.89	0.96	0.93
Histidine	1.52	1.70	1.61	1.49	1.58	1.54
Lysine	6.92	7.44	7.18	6.87	6.64	6.75
Total filtrate N	60.14	59.22	59.68	60.63	60.53	60.58
Amino	54.15	54.26	54.20	54.63	53.91	54.27
Non-amino	5.99	4.96	5.48	6.00	6.62	6.31
Total	100.83	100.42	100.60	100.57	100.36	100.47

each of which has merits. Being unable to decide the relative value of the various methods of preparation a comparison of them was undertaken in the hope that the results would give a basis on which to make a choice. Accordingly a large uniform sample of commercially milled first patent unbleached flour was obtained and, from this, gliadin and glutenin were prepared by the following methods.

I. Osborne Method

In this preparation the method used was that outlined by Osborne (20). This is the oldest and most widely used procedure and therefore has the advantage that the results obtained can be compared with those of other investigators. In this and subsequently discussed preparations 1000 gm. of flour was mixed to a stiff dough with 600 cc. of tap water and allowed to stand under water for two hours at the end of which time the crude gluten was washed out under a slow stream of water from the tap. The gluten was then left in tap water for 16 hr. and finally ground in a meat chopper. In all preparations, the finely ground gluten was placed in a four-litre beaker with three litres of the dispersion medium and stirred continuously by means of motor-driven glass stirring rods for approximately five hours, allowed to settle

overnight, the supernatant liquid decanted, more of the dispersion medium added, and the process repeated until extraction or dispersion was complete. All solutions were clarified by use of the Sharples supercentrifuge at a speed of 38000-40000 r.p.m. Alcoholic solutions were concentrated *in vacuo* at a temperature below 35° C.

In Preparation I the first extraction was made with three litres of 70% ethyl alcohol, followed by four extractions each with two litres of alcohol. The fourth extract contained 0.014% nitrogen and was discarded. Thereafter five more extractions were made, each with one-litre portions of the 70% alcohol, in order to remove the last traces of gliadin. The tenth and final extract showed only a trace of nitrogen. The alcoholic solution was centrifuged and concentrated to a thick syrup which was poured into 10 litres of distilled water containing five grams of NaCl. The precipitate was redissolved in one litre of 70% alcohol and this solution was again concentrated to a syrup from which gliadin was precipitated as before. The third precipitation was made by pouring the syrup into 400 cc. of a mixture of two parts absolute alcohol and one part anhydrous ether. Thereafter the protein was extracted twice with the absolute alcohol-ether mixture, three times with anhydrous ether, and finally dried

TABLE III

ANALYTICAL DATA OBTAINED FOR GLIADIN AND GLUTENIN PREPARED BY METHOD I (OSBORNE'S METHOD)

Analysis

	Gliadin	Glutenin
Amount recovered, gm.	28	10
Moisture, %	3.25	2.50
Ash, %	0.17	0.50
Nitrogen, %	16.36	15.83
Nitrogen (corr. for moist. and ash), %	16.93	16.84

Nitrogen distribution as determined by the Van Slyke method, %

Nitrogen fraction	Gliadin			Glutenin		
	1	2	Mean	1	2	Mean
Ammonia N	25.45	25.50	25.48	12.51	12.90	12.70
Total humin N	0.88	0.81	0.84	2.01	2.06	2.04
Acid insoluble	0.23	0.23	0.23	0.74	0.83	0.78
Acid soluble	0.24	0.22	0.23	0.84	0.74	0.79
Phosphotungstic	0.41	0.36	0.38	0.43	0.49	0.47
Total basic N	10.69	10.76	10.72	23.62	23.97	23.79
Arginine	5.23	5.22	5.22	12.81	12.50	12.65
Cystine	0.48	0.50	0.49	0.44	0.45	0.44
Histidine	4.32	4.35	4.33	6.89	7.33	7.11
Lysine	0.66	0.69	0.68	3.48	3.69	3.59
Total filtrate N	62.22	62.28	62.25	61.03	60 88	60.95
Amino	52.53	52.10	52.32	51.74	52.90	52.32
Non-amino	9.69	10.18	9.93	9.29	7.98	8.63
Total	99.24	99.32	99.29	99.17	99.81	99.49

in vacuo at 35° C. for 12-15 hr. It was then ground in a ball mill, re-dried *in vacuo* as a powder and after removal was carefully stoppered.

Glutenin was prepared by dissolving the residue from the gliadin extraction in 0.025 *N* NaOH. This solution was supercentrifuged and treated with dilute hydrochloric acid until maximum flocculation occurred. The precipitate was collected on a linen filter cloth and extracted with one litre of 70% alcohol after which it was redispersed in 0.025 *N* NaOH, centrifuged and precipitated. Two reprecipitations were made. The final precipitate was washed once with 95% alcohol, twice with absolute alcohol-ether mixture, and three times with anhydrous ether. It was then dried *in vacuo* at 35° C. for 12-15 hr., ground, re-dried, and stoppered.

Van Slyke analyses in duplicate were made on these preparations, according to the usual procedure. The data are given in Table III.

II. Osborne Method Without Dispersal of the Glutenin in any Solvent

In this preparation the gliadin was isolated as in the previous method. It was proposed to make a combination of the Osborne method and the Blish and Sandstedt acetic acid method, by extracting the gliadin with 70% alcohol and dispersing the residue in acetic acid in place of sodium hydroxide solution.

TABLE IV

ANALYTICAL DATA OBTAINED FOR GLIADIN AND GLUTENIN PREPARED BY METHOD II (NO DISPERSION OF THE GLUTENIN)

Analysis

	Gliadin	Glutenin
Amount recovered, gm.	40	33
Moisture, %	3.35	2.84
Ash, %	0.06	0.25
Nitrogen, %	16.39	11.86
Nitrogen (corr. for moist. and ash), %	16.97	12.23

Nitrogen distribution as determined by the Van Slyke method, %

Nitrogen fraction	Gliadin			Glutenin		
	1	2	Mean	1	2	Mean
Ammonia N	25.66	25.58	25.62	18.42	18.39	18.40
Total humin N	0.82	0.76	0.79	2.62	2.46	2.54
Acid insoluble	0.22	0.24	0.23	1.53	1.71	1.62
Acid soluble	0.25	0.12	0.18	0.69	0.45	0.57
Phosphotungstic	0.35	0.40	0.38	0.40	0.30	0.35
Total basic N	10.74	10.27	10.50	18.59	17.62	18.10
Arginine	5.44	5.57	5.50	8.45	8.74	8.59
Cystine	0.62	0.73	0.67	0.72	0.79	0.76
Histidine	4.05	3.34	3.70	4.84	3.79	4.31
Lysine	0.63	0.63	0.63	4.58	4.30	4.44
Total filtrate N	61.81	62.64	62.23	61.00	61.48	61.24
Amino	52.51	52.54	52.53	53.43	54.03	53.73
Non-amino	9.30	10.10	9.70	7.57	7.45	7.51
Total	99.03	99.25	99.14	100.63	99.95	100.28

It was found, however, that when 0.07 N acetic acid was added no apparent dispersion took place. The residue absorbed the solution and swelled until it completely filled the four-litre container with a jelly-like mass. This was transferred to a 14-litre vessel and seven litres more acetic acid solution was added. After continuous stirring for five hours, the solution showed 0.011% nitrogen or approximately six grams of protein dissolved in the 10 litres. Various concentrations of the acid solution were tried with small portions of the material but dispersion could not be effected. The mass was drained on a linen filter and then washed three times with eight-litre portions of distilled water with very vigorous stirring in an effort to separate by mechanical means as much starch as possible. As the residue was still exceedingly bulky $N/14$ NaOH solution was carefully added until the solution remained just faintly acid, the volume of the residue being thus reduced from about four litres to 300 cc. This was then reground in the meat chopper and washed with continuous stirring in three successive portions of 10 litres of distilled water. It was finally drained and dried in the usual manner.

Analyses of these two proteins are given in Table IV. The glutenin was very low in nitrogen, the value being 12.23%, which indicates that it probably contained a high percentage of starch, probably about 28%. No work has been done to determine the effect on the nitrogen distribution of this concentration of starch. Hart and Sure (7) carried out the Van Slyke analysis on a mixture of 2.4 gm. casein and 12 gm. starch but this represents a very much greater degree of contamination than in the sample in question here. On consideration of the available data regarding the effect of carbohydrates on the nitrogen distribution Larmour (15) concluded that the humin nitrogen and filtrate nitrogen are most affected and that the basic fractions may be regarded as fairly reliable.

In the glutenin of Method II the humin nitrogen shows an average value of 2.54% which, compared to the value 2.04% for glutenin by Osborne's method, would indicate that the impurity was not having a great effect on the distribution, as the humin nitrogen fraction is the one that is most profoundly affected

TABLE V

Comparison of data obtained with duplicate preparations of gliadin made by the Osborne method

Nitrogen fraction	Preparation I			Preparation II			Diff. of means I-II
	1	2	Mean	1	2	Mean	
Ammonia N	25.45	25.50	25.48	25.66	25.58	25.62	−0.14
Total humin N	0.88	0.81	0.84	0.82	0.76	0.79	0.05
Total basic N	10.69	10.76	10.72	10.74	10.27	10.50	0.22
Arginine	5.23	5.22	5.22	5.44	5.57	5.50	−0.28
Cystine	0.48	0.50	0.49	0.62	0.73	0.67	−0.18
Histidine	4.32	4.35	4.33	4.05	3.34	3.70	0.63
Lysine	0.66	0.69	0.68	0.63	0.63	0.63	0.05
Total filtrate N	62.22	62.28	62.25	61.81	62.61	62.23	0.02
Amino	52.53	52.10	52.32	52.51	52.54	52.53	−0.21
Non-amino	9.69	10.19	9.93	9.30	10.10	9.70	0.23
Total	99.24	99.32	99.29	99.03	99.25	99.14	

by carbohydrates. It is quite probable that this relatively low percentage of carbohydrate did not materially alter the nitrogen distribution particularly with respect to the basic fractions.

The gliadin preparations in Methods I and II were prepared by the same technique, therefore the analytical data for the two samples may be used to examine the agreement of duplicate preparations of this protein by the Osborne method. For convenience in making comparison the data are reproduced in Table V. Inspection of these data shows that the differences between all four analyses are no greater than would be found in four replicate analyses of one individual preparation. It is evident, therefore, that only very small errors may be expected in replicate preparations of gliadin by the Osborne method.

III. Method of Blish and Sandstedt (2)

This method differs from Osborne's in that the crude gluten is initially all dispersed in dilute sodium hydroxide solution. The solution is then treated with 95% alcohol until the alcoholic strength reaches 70% and from this the glutenin is precipitated by adding acid until the isoelectric pH of glutenin is reached. The gliadin is recovered from the filtrate in the usual manner. This method has the advantage that it is a more rapid one and does not involve long exposure to alcohol.

TABLE VI

ANALYTICAL DATA OBTAINED FOR GLIADIN AND GLUTENIN PREPARED BY METHOD III, (ALL GLUTEN DISPERSED IN ALKALI)

Analysis

	Gliadin	Glutenin
Amount recovered, gm.	21	13
Moisture, %	1.62	2.18
Ash, %	0.25	0.41
Nitrogen, %	16.50	16.07
Nitrogen (corr. for moist. and ash), %	16.82	16.50

Nitrogen distribution as determined by the Van Slyke method, %

Nitrogen fraction	Gliadin			Glutenin		
	1	2	Mean	1	2	Mean
Ammonia N	24.99	25.18	25.05	15.26	14.85	15.06
Total humin N	0.82	1.03	0.92	1.60	1.68	1.64
Acid insoluble	0.30	0.44	0.37	0.66	0.73	0.69
Acid soluble	0.29	0.41	0.35	0.59	0.64	0.62
Phosphotungstic	0.23	0.18	0.21	0.35	0.31	0.33
Total basic N	14.39	13.95	14.17	21.02	20.36	20.69
Arginine	6.01	6.29	6.15	11.91	11.60	11.75
Cystine	0.62	0.67	0.64	0.68	0.72	0.70
Histidine	6.17	5.36	5.77	3.87	3.32	3.60
Lysine	1.59	1.63	1.61	4.56	4.72	4.64
Total filtrate N	59.86	60.41	60.13	62.47	62.49	62.48
Amino	55.31	53.54	54.42	54.63	53.91	54.27
Non-amino	4.55	6.87	5.71	7.84	8.58	8.21
Total	100.06	100.57	100.27	100.35	99.38	99.86

Three litres of 0.025 N NaOH was used in the initial treatment. Most of the gluten dispersed leaving only a small residue which was extracted with two successive one-litre portions of sodium hydroxide solution. The third extract contained 0.015 gm. nitrogen per 100 cc. These last two solutions were concentrated *in vacuo* at 35° C. to a volume of 55 cc. and added to the first extract. The total solution, or more properly, dispersion, was centrifuged at 39000 r.p.m. at the slowest rate of feeding, and then made up to a concentration of 70% by addition of the necessary amount of 95% ethyl alcohol. Hydrochloric acid was added until the point of maximum flocculation was reached. The filtrate was centrifuged before concentration in order to remove any glutenin that had not settled out or that had escaped the linen filter cloth. Purification and drying of the proteins was carried out in the manner described in Method I. Analytical data are given in Table VI.

IV. Blish and Sandstedt (3) Method with 0.007 N Acetic Acid

There is much evidence to show that changes in the chemical properties of proteins occur when they are exposed to alkali. Knaggs and Schryver (13, 14) and later Knaggs (12) reported the observation that the diamino nitrogen fraction of gelatin varied according to the treatment given the collagen from which it was prepared. After a 60-day exposure to alkali, treatment with 0.5% HCl for one day prior to preparation of the gelatin produced a significant lowering of the diamino fraction. Blish and Sandstedt (3) showed that the basic nitrogen fraction of glutenin inversed with increasing strength of the alkali solution. This increase was accompanied by a decrease of ammonia nitrogen. On account of this variability in chemical composition of the glutenin prepared in alkaline medium they recommend a method of preparation in which dilute acetic acid is used as the dispersion medium in place of sodium hydroxide solution.

They state that difficulty was encountered in attempting to effect acid dispersion of gluten but that this was accomplished in very dilute acetic acid. Unfortunately they did not specify the strength of acid used. Rather empirically a concentration of 0.007N acetic acid was chosen and the ground wet gluten was treated with three litres. After five hours stirring there resulted a rather viscous milky dispersion which, on standing overnight, was little clearer. There was, however, a ¼-in. layer of residue left on the bottom of the vessel. The supernatant liquid was decanted and centrifuged. At the lower end of the centrifuge bowl there was found the usual deposit of fairly dry material characteristic of starch removal, but at the upper end there was quite a large amount of impure protein. This was separated and returned to the extraction vessel and treated with two litres of the acid. This extracted only a small fraction of the residue which, after a third treatment, was discarded although it was shown to contain some protein.

Blish and Sandstedt (3) remarked that a satisfactory separation of glutenin could not be made when ethyl alcohol was used in place of methyl alcohol. Two 100-cc. portions of the acetic acid-protein solution were made up to 70% alcohol by addition of ethyl and methyl alcohol respectively. Upon almost

complete neutralization by means of $N/14$ NaOH no differences in the rate of precipitation or in the nature of the precipitate obtained could be observed; therefore ethyl alcohol was used subsequently. This precipitation of glutenin is more difficult than in the other methods, due perhaps to the fact that the coagulum forms somewhat more slowly and there is a tendency to go past the optimum pH. However, at the correct pH there appears to be a very complete separation leaving practically a water-clear filtrate. The glutenin so obtained showed characteristics similar to those observed by Blish and Sandstedt (3), *i.e.*, it was more coherent and rubbery than other preparations and resembled the original gluten a great deal, although the tendency to coalesce was less marked than in gluten. In proceeding to purify this initial precipitate it was found that it would not dissolve in 0.007 N acetic acid to any appreciable extent. It was, therefore, ground in the chopper and extracted several times with 70% ethyl alcohol and finally dehydrated and ground in the usual manner. The gliadin was prepared from the filtrate in the usual manner and presented no exceptional difficulties.

TABLE VII

ASH, MOISTURE, AND NITROGEN OF REPLICATE PREPARATIONS BY METHOD IV, BLISH AND SANDSTEDT'S (1929) ACETIC ACID DISPERSION (USING 0.007 N ACETIC ACID)

	Gliadin		Glutenin	
	Prep. A	Prep. B	Prep. A	Prep. B
Amount obtained, gm.	32	31	16	12
Moisture, %	3.58	3.69	6.17	5.94
Ash, %	0.19	0.36	0.43	0.97
Nitrogen, %	16.33	16.22	16.08	15.97
Nitrogen, % (corrected for ash and moisture)	16.96	16.91	17.20	17.15

Replicate preparations were made by this method because it was thought that failure to dissolve all of the crude gluten might introduce appreciable errors in composition of the glutenin. The data for the Van Slyke analyses of the replicates are given in Table III and have been discussed from the standpoint of error involved in replication. The results appear to be quite reproducible by the method used here. Data for ash, moisture, and nitrogen which were omitted from Table III are shown in Table VII. These two samples of glutenin were higher in nitrogen than any of the other preparations made.

V. Blish and Sandstedt (3) Method, Using Acetic Acid 0.07 *N* in Place of 0.007 *N*

In this preparation 0.07 N acetic acid was used to disperse the wet crude gluten. A greater fraction of it actually dispersed but even after five successive treatments there was left a small residue from which protein could be extracted with 0.025 N NaOH. The quantity obtained by this latter treatment was much too small for analysis.

The glutenin precipitated from the acetic acid-70% alcohol solution was much less coherent and elastic than that obtained with use of 0.007 N acetic acid, and resembled more nearly the preparations from alkaline dispersions.

About 75% of this glutenin could be redispersed in 0.07N acetic acid but the remainder proved particularly resistant to acid dispersion. The reprecipitation of the glutenin was very difficult and only a small fraction was finally obtained. Without further attempts at dispersion the material was treated several times with 70% alcohol and finally dried in the usual way.

The gliadin gave some difficulty because in the initial precipitation it failed to coagulate and remained as a fine milky suspension. It was found necessary to neutralize most of the acetic acid left, and to increase the amount of sodium chloride to 20 gm. per 10 litres. Under these conditions a considerable amount was precipitated. Part of the gliadin still suspended was recovered from the upper part of the centrifuge bowl after the filtrate had been slowly fed through

TABLE VIII

ANALYTICAL DATA OBTAINED FOR GLIADIN AND GLUTENIN PREPARED BY METHOD V (DISPERSION IN 0.07 N ACETIC ACID)

Analysis

	Gliadin	Glutenin
Amount recovered, gm.	18	4
Moisture, %	1 48	4.14
Ash, %	0 31	0.59
Nitrogen, %	16.35	15.29
Nitrogen (corr. for moist. and ash), %	16 65	16 05

Nitrogen distribution as determined by the Van Slyke method, %

Nitrogen fraction	Gliadin 1	Gliadin 2	Gliadin Mean	Glutenin*
Ammonia N	25.40	24.86	25.13	16.07
Total humin N	1.03	1.01	1 02	2.14
Acid insoluble	0.47	0.47	0 47	0.77
Acid soluble	0.35	0.33	0 34	1.12
Phosphotungstic	0.21	0.21	0.21	0.25
Total basic N	13.84	14 47	14.15	20.50
Arginine	6.33	6.11	6.22	12.12
Cystine	1.05	1.03	1.04	0.63
Histidine	5 22	6 12	5 67	3.14
Lysine	1.24	1.21	1.22	4.61
Total filtrate N	59.34	60.35	59.85	61.48
Amino	52.65	52 57	52 61	55.30
Non-amino	6.69	7 78	7.24	6.18
Total	99 61	100.69	100.15	100.19

**As but 4 gm. of this preparation was obtained the values reported are for a single determination.*

the machine at 40000 r.p.m. From a Kjeldhal determination made on the filtrate it was estimated that about 20 gm. of gliadin was lost at this point. The data, in Table VIII, show that the gliadin was lower in nitrogen than any of the other preparations of this protein and, excepting Preparation II, this holds for the glutenin also.

Discussion of the Results

In order to facilitate comparison of all the results obtained, the mean values of analyses on the various preparations have been brought together in Table IX. One is impressed immediately with the uniformity of results obtained with the gliadin. Were it not for the variations in the total basic and non-amino filtrate fraction, the analyses exhibit no greater variation than might be expected from replicate analyses of one preparation. The range in nitrogen

TABLE IX

SUMMARY OF ANALYSES MADE ON THE VARIOUS PREPARATIONS

Method of preparation	N in protein	Ammonia N	Humin N	Total basic N	Arginine N	Cystine N	Histidine N	Lysine N	Filtrate N	
									Amino	Non-amino
Gliadin										
I. Osborne's method	16.9	25.5	0.8	10.7	5.2	0.5	4.3	0.7	52.3	9.0
II. Osborne's method	17.0	25.6	0.8	10.5	5.5	0.7	3.7	0.6	52.5	9.7
III. Blish and Sandstedt (1924)	16.8	25.0	0.9	14.2	6.2	0.6	5.8	1.6	54.4	5.7
IV. Blish and Sandstedt (1929) acetic acid 0.007 *N* Prep. 1	17.0	25.8	0.8	10.5	5.4	0.8	3.8	0.6	53.5	9.3
Prep. 2	16.9	25.7	0.8	11.1	5.2	0.6	4.6	0.7	53.9	8.1
V. Blish and Sandstedt (1929) acetic acid 0.07 *N*	16.6	25.1	1.0	14.2	6.2	1.0	5.7	1.2	52.6	7.2
Glutenin										
I. Osborne's method	16.8	12.7	2.0	23.8	12.6	0.4	7.1	3.6	52.3	8.6
II. Osborne's method	12.2	18.4	2.5	18.1	8.6	0.8	4.3	4.4	53.7	7.5
III. Blish and Sandstedt (1924)	16.5	15.1	1.6	20.7	11.8	0.7	3.6	4.6	54.3	8.2
IV. Blish and Sandstedt (1929) acetic acid 0.007 *N* Prep. 1	17.2	21.4	1.4	18.2	8.4	0.9	1.6	7.2	54.2	5.5
Prep. 2	17.2	20.6	1.5	17.8	8.6	0.9	1.5	6.8	54.3	6.3
V. Blish and Sandstedt (1929) acetic acid 0.07 *N*	16.1	16.1	2.1	20.5	12.1	0.6	3.1	4.6	55.3	6.2

was 16.6-17.0%, the low value being for gliadin by Method V (0.07 *N* acetic acid). The other five preparations showed a variation of 0.2%. The range for ammonia nitrogen was 25.0% to 25.8% which was within the limits of experimental error. The total humin nitrogen was quite constant, indicating that carbohydrate contamination was of approximately the same extent in all cases. The total basic nitrogen in Preparations III (gluten initially dispersed in alkali) and V (0.07 *N* acetic acid) was approximately 40% higher than in the others. This was made up of increases in arginine, histidine, and lysine nitrogen. These two preparations also had the lowest ammonia-nitrogen fraction and the lowest non-amino filtrate nitrogen. Furthermore, these two analyses checked as closely as duplicate determinations on the same sample. It appears that prolonged exposure to 0.025 *N* NaOH or to 0.07 *N* acetic acid produces practically the same sort of modification of the nitrogen distribution. Haugaard and Johnston (8) have shown that gliadin can be fractionated on the basis of solubility and that the most readily soluble fraction is highest in non-amino nitrogen and lowest in ammonia nitrogen. As the

preparations under discussion are lowest in both ammonia nitrogen and non-amino nitrogen, the differences observed cannot be attributed to fractionation on the basis of solubility in the different media employed.

Considering the glutenins, it is evident that the preparations made by Method IV, using 0.007 *N* acetic acid, were the purest. They were highest in nitrogen and lowest in humin nitrogen. The only two preparations that came at all close to checking in respect to ammonia and basic nitrogen are III (alkali dispersion of the gluten) and V (using 0.07 *N* acetic acid). Except for non-amino filtrate nitrogen and, to slighter extent, ammonia nitrogen, these might be taken for replicates. This tends to confirm the conclusion reached in regard to gliadin, namely, that long exposure to alkali and exposure to acid as strong as 0.07 *N* acetic acid tend to produce the same sort of variation.

There is a great range of variation in ammonia nitrogen, total basic nitrogen, and arginine nitrogen in these preparations. The Osborne method gave low ammonia nitrogen and high basic nitrogen, while Method IV (0.007 *N* acetic acid) gave high ammonia nitrogen and low basic nitrogen. It is interesting to note that the relation of ammonia nitrogen and basic nitrogen in these preparations, with the exception of Preparation IV, were virtually interdependent so that one varied inversely as the other. This is brought out clearly in Table X. Thus in four of the five preparations the sum of these two fractions was almost

TABLE X

THE RELATIONSHIP OF AMMONIA NITROGEN AND TOTAL BASIC NITROGEN IN THE GLUTENIN PREPARATIONS

Preparation	Ammonia N	Total Basic N	Sum
I	12.7	23.8	36.5
II	18.4	18.1	36.5
III	15.1	20.7	35.8
IV (mean)	21.0	18.0	39.0
V	16.1	20.5	36.6

TABLE XI

VALUES FOR AMMONIA NITROGEN AND BASIC NITROGEN IN VARIOUS GLUTENIN PREPARATIONS

Observer	Source of flour	Method	Ammonia N	Basic N	Sum
Blish (1)	Spring wheat	Alkali	16.5	18.0	34.5
Blish	Soft wheat	Alkali	16.2	18.9	35.1
Cross and Swain (5)	Idaho wheat	Alkali	15.6	22.6	38.2
Cross and Swain	Patent	Alkali	16.0	23.0	39.0
Cross and Swain	Club	Alkali	14.2	26.0	40.2
Cross and Swain	Forty-fold	Alkali	13.1	26.2	39.3
Hoffman and Gortner (10)	Patent	Alkali	13.6	21.9	35.5
Larmour (15)	Patent	Alkali	14.8	18.8	33.6
Csonka and Jones (6)	(α-glutelin)	Alkali	17.8	21.3	39.1
Csonka and Jones	(β-glutelin)	Alkali	11.1	24.6	35.7
Cook and Alsberg (4)	Spring wheat	Alkali	17.9	18.2*	36.1
Cook and Alsberg	Spring wheat	Urea	16.4	18.7*	35.1
Cook and Alsberg	Spring wheat	Urea (4 preps.)	19.5	14.5*	34.0
Authors Prep. I	Spring wheat	Alkali	12.7	23.8	36.5
Authors Prep. II	Spring wheat	No dispersion	18.4	18.1	36.5
Authors Prep. III	Spring wheat	Alkali	15.1	20.7	35.8
Authors Prep. IV	Spring wheat	Acetic acid (0.007 *N*)	21.0	18.0	39.0
Authors Prep. V	Spring wheat	Acetic acid (0.07 *N*)	16.1	20.5	36.6

* *Without the correction for arginine in the filtrate from the bases.*

constant. This would indicate that if by any manipulation the ammonia nitrogen were decreased, the basic nitrogen would be increased by the same amount. This is admirably shown in Blish and Sandstedt's (3) data on the influence of alkali of different strengths. Their Tables III and IV show that the decrease in amide nitrogen is almost exactly equal to the corresponding increase in basic nitrogen. It should be noted that no change occurs in filtrate nitrogen comparable to the changes in ammonia and basic nitrogen. That this is not a fortuitous relationship is indicated by the data of a number of investigators given in Table XI. The correlation coefficient $r = -0.84$ indicates a very marked tendency for the basic nitrogen to vary inversely as the ammonia nitrogen.

TABLE XII

THE RELATIONSHIP OF AMMONIA NITROGEN AND TOTAL BASIC NITROGEN IN THE GLIADIN PREPARATIONS

Preparation	Ammonia N	Basic N	Sum
I	25.5	10.7	36 2
II	25.6	10 5	36.1
III	25 0	14.2	39.2
IV (mean)	25.8	10.8	36.6
V	25 1	14.2	39.3
			Mean 37.5%

Furthermore, attention should be directed to the fact, shown in Table XII, that the sum of these two fractions in gliadin tends to approach the same value as in glutenin. The mean value of the sum for the glutenins in Table XI is 37.0% and for the gliadins in Table XII it is 37.5%. From all analyses available on wheat gliadin we obtain 38.0% as the mean value of the sum of ammonia- and basic nitrogen. It seems probable, therefore, that this sum may be a more important constant than either of its components.

The explanation of this relation between these two fractions that probably first comes to mind is that, in the course of treatment of the protein with alkali, ammonia is lost. If this occurred it would lower the nitrogen content of the protein and increase the percentage of all but the ammonia fraction. It will be observed that in this work Preparation IV, having the highest nitrogen in the protein, had the highest ammonia nitrogen fraction, but no definite conclusion on this basis can be reached as we have no means for accurately estimating the carbohydrate impurities present in the various preparations. Moreover, it can be shown by calculation that the theoretical change wrought in the distribution of the fractions does not correspond to the experimental facts. Taking, as a case, the glutenin preparation of highest ammonia nitrogen, namely, Preparation I of Method IV, and assuming that it represents the real protein, suppose that in course of isolation it lost 54.7% of its ammonia nitrogen. The modified protein would then, upon analysis, give the nitrogen distribution shown in column 2 of Table XIII. Thus if one attempts to explain the difference between the glutenins of Method IV and Method I, by assuming a loss of 54.7% of the ammonia nitrogen due to action of the alkaline solution, the theoretical distribution calculated on the basis of such a loss does not agree with the results as actually found with a sample of glutenin

having 12.7% ammonia nitrogen. In place of 19.8% basic nitrogen calculated there was found 23.8%, and in place of 66 0% filtrate nitrogen calculated, 60.9%.

TABLE XIII

CALCULATIONS SHOWING THE EFFECT ON THE NITROGEN DISTRIBUTION OF GLUTENIN (AVERAGE, METHOD IV) OF A LOSS OF 54.7% OF ITS AMMONIA NITROGEN

Original		After loss of 46.4% of its ammonia nitrogen, %	Nitrogen distribution of glutenin by Osborne's method, %
Ammonia N	21 0	12.7	12.7
Humin N	1 4	1.5	2.0
Basic N	18.0	19.8	23.8
Filtrate N	60 1	66 0	60.9

As will be shown later there is loss of nitrogen as ammonia when glutenin is treated with alkali, but this alone cannot account for the increase in basic nitrogen. Leaving aside glutenin IV, the others all show increase of basic nitrogen commensurate with increase of ammonia nitrogen and relatively slight variation in filtrate nitrogen. Loss of nitrogen in course of isolation of the protein will not therefore explain the differences in nitrogen distribution noted in Preparations I, II, III, and V.

On the other hand, if it is true that the ammonia nitrogen and basic nitrogen fractions are inversely proportional, a new interpretation of the ammonia nitrogen must be found. If this fraction is from amide groupings only, it is difficult to see why a treatment resulting in lowered ammonia nitrogen should produce a corresponding increase in the basic fraction. Without more data it is rather useless to speculate concerning the reason for this remarkable relationship. It seems fairly evident, however, that much weight should not be attached to either ammonia nitrogen or total basic nitrogen of preparations made by methods involving use of alkali or of 0.07 N acetic acid.

TABLE XIV

RESULTS OBTAINED BY TREATING GLUTENIN OF METHOD IV (BLISH AND SANDSTEDT'S (1929) ACETIC ACID METHOD) FIRST WITH 0.1% SODIUM HYDROXIDE FOR ONE WEEK AND SUBSEQUENTLY HYDROLYZING AND FRACTIONATING THE RESIDUE

Fraction	Original values of glutenin of Method IV (Prep. A)	Values obtained on treated sample as % of initial N	Values calculated on basis of 4.8% N lost in initial treatment
N removed by treatment with 0.1% NaOH		4.80	
Ammonia N after acid hydrolysis	21.38	16.63	17.47
Total ammonia N		21.43	
Humin N	1.40	1.41	1.48
Total basic N	18.16	17.99	18.90
Arginine N	8.43	8.05	8.46
Sum of ammonia N and basic N	39.54		36.37

In order to ascertain if ammonia is lost when a protein is exposed to alkali, 3 gm. of glutenin of Preparation IV (0.007 N acetic acid) was dissolved in 250 cc. of 0.025 N NaOH in a Claissen flask which was connected up as for the ammonia nitrogen determination. This was left, protected by toluene for

one week, at the end of which time it was distilled *in vacuo* at 40° C. It was found that 4.81% nitrogen was removed by this treatment. The residue was neutralized, the solution evaporated and the protein hydrolyzed in the usual manner. Results of the analysis are shown in Table XIV. In the third column of Table XIV are shown the values calculated to the basis of the nitrogen content of the protein after it had been submitted to cold digestion with 0.025 *N* NaOH for seven days. There was a marked decrease in ammonia nitrogen and a slight increase in basic nitrogen. By reference to column 2 it is evident that with respect to the initial protein these values are all spurious, as the value of the ammonia fraction appears to be increased, whereas in reality there was virtually no change in it attributable to the treatment by 0.025 *N* NaOH. It should be noticed too that the sum of ammonia- and basic-nitrogen has been reduced from 39.54 to 36.37%, the latter value being comparable to that obtained for the other four preparations in the series (see Table X).

Comparing the first and third columns of Table XIV it can be seen that the reduction in the ammonia-nitrogen fraction is not accompanied by a corresponding increase in the basic nitrogen fraction as was observed by Blish and Sandstedt (3) in their experiments with alkali of different strengths. In this experiment it appears that the only effect of the digestion in alkali was a loss of nitrogen from the protein. There was no significant change in the basic fraction. The initial loss of nitrogen, however, caused a redistribution of percentages for the remaining nitrogen, resulting in values that were spurious in respect to the original protein. Unfortunately only one concentration of alkali was used and we have no information concerning the effect of different strengths of sodium hydroxide on the distribution in column 3.

The question of choice of the five methods used seems to be quite definitely settled by this experiment. The protein prepared by Method IV, having a high initial ammonia-nitrogen fraction,loses nitrogen when brought into contact with alkali of the strength ordinarily used. This is doubtless a loss of ammonia nitrogen because the basic fraction does not seem to be affected. The loss of a little ammonia nitrogen from a protein would probably not be a serious matter by itself, especially as we do not know the origin of this fraction, but the resulting redistribution of all the other fractions constitutes an important source of error particularly as there is some evidence for believing that the amount of nitrogen lost from a protein is proportional to the time of exposure to the alkaline solution. The time during which a protein is kept in solution is most difficult to control because it depends so much upon the operator's success in making filtrations, or upon the operation of the centrifuge, or upon the speed with which the isoelectric point can be reached in making the precipitation. The use of alkaline solutions in preparing glutenin thus introduces possibilities of wide errors in the final analyses and, therefore, we endorse Blish and Sandstedt's (3) conclusion that it should be strictly avoided. On the other hand use of 0.07 *N* acetic acid gives results similar to those obtained in preparations

involving alkali. Of the five methods applied in this study Method IV, involving use of 0.007 N acetic acid, seems to be the best.*

References

1. Blish, M. J. J. Ind. Eng. Chem. 8: 138-144. 1916.
2. Blish, M. J. and Sandstedt, R. M. Cereal Chem. 2: 57-67. 1925.
3. Blish, M. J. and Sandstedt, R. M. J. Biol. Chem. 85: 195-206. 1929.
4. Cook, W. H. and Alsberg, C. L. Can. J. Research, 5: 355-374. 1931.
5. Cross, R. J. and Swain, R. E. J. Ind. Eng. Chem. 16: 49-52. 1924.
6. Csonka, F. A. and Jones, D. B. J. Biol. Chem. 73: 321-329. 1927.
7. Hart, E. B. and Sure, B. J. Biol. Chem. 28: 241-249. 1916.
8. Haugaard, G. and Johnston, A. H. Compt. rend. Trav. Lab. Carlsberg 18: 1-138. 1930.
9. Hayes, H. K., Immer, F. R. and Bailey, C. H. Cereal Chem. 6: 85-96. 1929.
10. Hoffman, W. F. and Gortner, R. A. Colloid Symposium Monograph, 2: 209-368. 1925.
11. Hoffman, W. F. and Gortner, R. A. Cereal Chem. 4: 221-229. 1927.
12. Knaggs, J. Biochem. J. 23: 1308-1327. 1929.
13. Knaggs, J. and Schryver, S. B. Biochem. J. 18: 1095-1101. 1924.
14. Knaggs, J. and Schryver, S. B. Biochem. J. 18: 1102-1106. 1924.
15. Larmour, R. K. J. Agr. Research, 35: 1091-1120. 1927.
16. Larmour, R. K. Trans. Roy. Soc. Can. V, 22: 349-363. 1928.
17. Larmour, R. K. Cereal Chem. 7: 35-48. 1930.
18. Larmour, R. K. Cereal Chem. 8: 179-189. 1931.
19. Mangels, C. E. Cereal Chem. 3: 150-157. 1926.
20. Osborne, T. B. The proteins of the wheat kernel, Carnegie Inst. Pub. 84. 1907.
21. Zinn, J. J. Agr. Research, 23: 529-548. 1923.

**Recently Cook and Alsberg (4) have published an account of results obtained with glutenin prepared in neutral 30% urea solutions. Using the sulphydryl test as a criterion they concluded that less denaturation occurs by use of urea solutions than when alkali or acid are used. The ammonia nitrogen values in their preparations are somewhat lower than in our preparation by Method IV using 0.007 N acetic acid. This, however, cannot be used to compare the methods as the proteins were prepared from different samples of flour.*

THE UTILITY OF COOKED POTATO IN BAKING BREAD AND ITS RELATION TO CRUDE PROTEIN AND BAKING STRENGTH[1]

By R. H. Harris[2]

Abstract

Two commercially milled flours, baked by a formula including the liquid drained from boiled sliced potato, gave loaves showing a progressive increase in color and loaf volume with increasing quantities of the liquid Dried mashed potato, containing all the original material, also caused an increase in volume with each increment of potato.

A number of doughs, including controls and doughs treated with varying proportions of mashed potato, showed increased gas production with increasing quantities of potato. The gas lost from the doughs also tended to increase in the same manner but was less than the increase in the total gas evolved. The volume of the doughs accordingly increased with increasing potato concentration.

A series of 10 commercial flours of various types was baked by the simple basic formula and by one including 5, 10 and 40% of cooked mashed potato in addition. A baking was also made of a blend of 50 gm. of flour to 50 gm. of potato and another using 1% diastatic malt and 0.001% $KBrO_3$ in addition to the simple ingredients. The resultant loaf volumes were found to increase as more potato was added. The higher protein flours gave larger loaves throughout. The color of the loaves decreased with the higher potato concentrations, the grain and texture of these loaves also being very poor. Loaf volumes and baking score, calculated on a basis of 100 gm. total material, decreased above 30% potato concentration. Loaves baked with more than 10% potato to 100 gm. flour were of inferior grain and texture. Crude protein and loaf volume were significantly related throughout.

Introduction

Cooked potato is extensively used in bread making by many people who are accustomed to produce all or part of their bread requirements. The liquid drained from the potato, "potato water," is frequently used and added to the yeast before mixing the dough or to the flour in place of ordinary water. In other cases, the mashed potato is mixed in with the flour and other ingredients, or a mixture of potato water and potato is used with either sponge or straight-dough methods with apparently beneficial results. This widespread use of cooked potato as a flour improver would seem to indicate some inherent virtue in this vegetable with respect to its influence on bread making in general.

Jago (7) mentions the use of cooked potato in various ways by Scottish and English bakers, and claims that substantial improvement is noted in the loaf when potato is included in the dough mix. He attributes the utility of the potato to the soluble nitrogenous material and dextrinized starch contributed to the dough and available as food for the yeast and its enzymes. The modern commercial baker, however, depends upon various manufactured flour improvers to get increased bread quality from his flour. These improvers possess the advantage of being available in a form very convenient for use. The only kind of potato employed appears to be a factory product in the form

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Contribution from the laboratories of the Quaker Oats Company, Saskatoon, Saskatchewan. Canada.

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of potato flour, which has a limited use for special breads and pastries.

To ascertain whether the use of potato as a flour improver was justified and to determine its effect upon the relation between crude protein and baking strength, the following work was carried out.

Materials and Methods

The flours used in this study were all of commercial type and were milled from the 1930 Western Canada crop with the exception of No. 1, which was produced from Ontario soft winter wheat. Flour No. 2 was milled from a blend containing a high percentage of Garnet and showed a rather dark loaf color. Several millstream flours, No. 3, 9 and 10, were included to obtain information regarding the bleaching effect of potato upon unbleached flour and to furnish samples in the higher protein range. Sample No. 8 was a strong first clear and produced a dark colored loaf of inferior grain and texture. Samples No. 4 and 5 were first patent flours while No. 6 and 7 were second patents.

The baking method was as follows: the doughs were mixed by hand in earthenware bowls (height, $4\frac{1}{2}$ in.; diameter, 5 in.; thickness of wall, $\frac{1}{4}$ in.) and run in pairs at intervals of five minutes. The Blish standard method (2) was followed in regard to fermentation, proofing time and temperature. The formula used was: flour, 100 gm.; yeast, 3 gm.; sugar (sucrose), 2.5 gm.; salt, 2 gm.; distilled water, as required for proper consistency. The salt and sugar solutions were added from a 100-cc. burette, 10 cc. being required for each dough. The water was measured in a 50-cc. burette supplied by syphon from a 1000-cc. reservoir. One of each pair of doughs was checked for temperature subsequent to mixing, and warm water added to the reservoir if the dough temperature became lower than 29° C. The absorption was varied to suit the requirements of each flour. The yeast suspension was measured in a 10-cc. graduated cylinder. The salt and sugar solutions used, as well as the yeast suspension, were corrected for volume of solute. This method is called the simple or basic procedure.

A modification of the simple procedure was also used, including the addition of 1% diastatic malt and 0.001% $KBrO_3$ to the basic ingredients. The malt was introduced as a solution, 4 cc. containing 1 gm. diastatic malt and 3.3 cc. of water. The potassium bromate was added by means of a pipette from a stock solution, 1 gm. to 1000 cc. distilled water. This variation of the standard or basic procedure is called the improver method in this investigation. It has been extensively used by Larmour and MacLeod (11), Geddes (4), Geddes and Goulden (5), and Harris (6). The utility of this method lies in the fact that it tends to bring out the inherent possibilities of a flour, which might escape detection when the basic method alone is used. The action of these ingredients is also apparently analogous to that of those added in the treatment of flour in a commercial bakery, where flour improvers are in common use.

Results

Effect of Cooked Potato

The simple basic formula plus the required quantity of potato (liquid or

mashed) was used in determining the effect of the cooked potato upon the baking properties of the flours. This ingredient was prepared as follows: the potato water was drained from peeled and sliced potatoes after boiling until the potatoes were thoroughly cooked. The liquid was quite opalescent especially at first, and deposited material of a starchy nature upon standing in a cool place. When incorporated in dough this deposited matter apparently possessed greater beneficial properties than the original liquid. Centrifuging had no appreciable effect upon the bread improving properties and did not remove the opalescence of the liquid. The potato water was concentrated by boiling at atmospheric pressure without changing its effect, but as the liquid became more viscous with increasing evaporation the color darkened rapidly, with resultant detriment to the loaf color. The stimulative action however persisted. After several attempts the concentration of the liquid by evaporation was abandoned and cooked mashed potato substituted for the potato drainings.

For this purpose, the potatoes were first peeled and all blemishes removed. The peeled potatoes were then washed in water and sliced into small pieces, sufficient tap water being added to insure thorough cooking of the vegetable before the water evaporated sufficiently to cause burning. Approximately 25 min. was required to thoroughly cook the potato. The cooking was done in a galvanized vessel without a lid, to assist evaporation of the water, thus obviating loss of soluble material from the potato through draining off any residual liquid. The potato was then quite soft and easily disintegrated by stirring vigorously with a broad spatula. The wet mass was dried at a slow heat for approximately one hour with frequent stirrings. It was found that drying for too long a period caused hard, lumpy masses to form, which were very difficult to break up and came through the baking process practically unchanged. The moist potato also appeared to yield better results.

The potato water was found to contain 0.03 gm. of nitrogen per 25 cc. The mashed potato contained 0.41% of nitrogen and 0.82% of ash, and had a high moisture content. The percentage of moisture in the potato was not determined, owing to the difficulty encountered in drying to constant weight.

The inorganic residue left after ashing the potato at dull redness was found to be without any great influence upon the baking properties of the flour. A concentration as high as 5% was used, leaving the loaf volume quite unaffected, but slightly darkening the color to a greyish tinge. The texture also appeared to be somewhat coarsened. From this, it would appear that the effect of potato cannot be ascribed to the presence of inorganic salts carried into the dough with the potato.

Experiments with Raw, Uncooked Potato

A trial was made, using raw, uncooked potato in place of the boiled. The potato in this case was peeled and finely grated before adding the flour. No attempt was made to dry the wet mass on account of the possible effect of heat upon the proteins and starch. No improvement was noted except in color and here the change was very slight. It is evident that heating causes a

change in potato proteins and starches, rendering them more suitable as nutrient material in the dough. The enzymes in potato do not appear to have any great effect upon the baking process, as the heating necessary in thoroughly cooking the potato would tend to inactivate them. To settle this point definitely, a portion of the potato water was evaporated to a dark brown paste and heated to the burning point (120-125° C.), then the paste was used with the simple formula in baking several loaves. No diminution in the improving action of the material upon loaf volume was noted.

The baking score was computed in the following manner:

Loaf volume...........	×0.1		
Symmetry.............	×1.0	Maximum value.........	10
Grain of loaf..........	×1.0	Maximum value.........	10
Color.................	×1.0	Maximum value.........	20
Texture...............	×1.0	Maximum value.........	10

The sum of these individual scores was considered the baking value.

Effect of Potato Water

Table I shows the baking data obtained on two commercial flours using varying quantities of potato water from 0 to 50 cc. A regular increase in

TABLE I

BAKING DATA OBTAINED WITH TWO FLOURS WHEN INCREASING QUANTITIES OF POTATO WATER WERE INCLUDED IN THE BASIC FORMULA

Flour No. 11							Flour No. 12						
Potato water, cc.	Volume, cc.	Symmetry	Color	Grain	Texture	Score	Potato water, cc.	Volume, cc.	Symmetry	Color	Grain	Texture	Score
0	470	9	20	10	10	96	0	480	7	16	8.5	9	88
10	520	10	22	10	11	105	10	578	8.5	17	8	8	99
20	570	10.5	22	9.5	10.5	109	20	620	9	17.5	8	9	105
30	590	11	23	9.5	9.5	112	30	630	10.5	18	8	9	108
40	598	11	22	9	9	111	40	645	11	18.5	7	8	109
50	615	11	19	8.5	9	109	50	660	10 5	19.5	7	7.5	110

loaf volume is noted with increasing concentration of potato water. The symmetry and color also tend to improve although there is some evidence of falling-off in color after 30 cc. in one case. The grain and texture scores of the loaf decrease with increasing potato water.

Effect of Higher Potato Concentration

To determine the effect of higher potato concentration upon loaf volume and color, the mashed potato was employed in concentrations of 1 to 60% on 100 gm. of flour, and also with different proportions of potato and flour up to 50 gm. of each, using 100 gm. of combined flour and potato. The baking data thus obtained is shown in Table II. A regular increase in loaf volume is shown with increasing quantities of potato, and when the values are calculated on 100 gm. of total material, an increase in loaf volume over the basic results

TABLE II

BAKING DATA OBTAINED BY INCLUDING INCREASING PERCENTAGES OF COOKED MASHED POTATO IN THE BASIC FORMULA. CORRECTED TO 13.5% MOISTURE BASIS

% Potato	Loaf volume, cc.		Symmetry	Color	Grain	Texture	Scores calculated using *a* and *b* loaf volumes	
	a	*b*						
Flour No. 13								
0	490	490	8	21	9	9.5	96	96
1	505	500	9	20.5	8.5	9	97	97
2	520	509	10	21	8	9	100	99
5	545	519	10.5	23	6	7	101	98
10	580	527	10.5	24	6.5	7	106	101
15	620	539	10.5	24	4	5	105	97
20	645	537	10	25	3	3	105	95
25	642	513	9.5	26	3	3	96	83
30	695	535	10.5	26	3	2	111	95
40	735	525	10	26	2	2	113	92
50	755	503	9	25.5	2	2	114	89
Flour No. 14								
0	490	490	8	22	9.5	9	97	97
1	482	477	8	22.5	10	10	98	98
2	512	502	9	23	10	10	103	102
5	528	503	9.5	24	8	8	102	100
10	585	532	9.5	23	7	7	105	100
15	590	513	10	24	6.5	7	106	99
20	620	517	10	23.5	5	5	105	95
25	660	528	10	24.5	5	5	110	97
30	670	515	10	24	4.5	5	110	95
40	680	485	10	24.5	4	5.5	112	92
50	725	483	10	22	3	4	115	87
60	750	468	10	21.5	2	3	111	83

NOTE: *a = measured loaf volume on 100 gm. flour; b = loaf volume calculated on 100 gm. of total material.*

is evident up to at least 30% of potato concentration. This behavior is also shown in the case of the bakings with a total of 100 gm. of combined potato and flour. An increase over the basic loaf volumes is shown up to a flour-potato ratio of 70:30, corresponding to 42.8% potato to 100 gm. of flour. The color score of the loaf also increases with increasing quantities of potato, reaching a maximum at about 30% potato, when the larger loaves are considered. In the case of the test with a total of 100 gm. of the material, the color in one instance becomes darker at the 50-50 concentration, but with the other flour the color does not fall even at this point.

The loaves baked with 100 gm. flour plus potato show progressive decrease in grain and color after the initial treatment with potato, becoming very poor for the higher concentrations. The same trend is evident, possibly in a less marked degree, with the other bakings. The bread produced above concentrations of 10% of potato would probably be considered unsatisfactory for commercial purposes. It was noticed that the bread made with potato

remained fresh and moist much longer than the basic loaves kept under the same conditions.

TABLE III

BAKING DATA OBTAINED BY INCLUDING INCREASING RATIOS OF COOKED MASHED POTATO TO FLOUR IN THE BASIC FORMULA

Ratio flour/potato	Potato as per cent of flour	Loaf volume, cc.		Symmetry	Color	Grain	Texture	Score
		a	*b*					
Flour No. 13								
100 Flour 0 Potato	0	490	490	8	21	9	9.5	96
90 Flour 10 Potato	11.1	540	600	9 5	22	9	9 5	104
80 Flour 20 Potato	25.0	520	650	10	23	7	7	99
70 Flour 30 Potato	42.8	510	729	8	24	4	4	98
60 Flour 40 Potato	66.6	434	723	7	25	4	4	84
50 Flour 50 Potato	100.0	365	730	4	25	3	2	70
Flour No. 14								
100 Flour 0 Potato	0	490	490	8	22	9 5	9	97
90 Flour 10 Potato	11.1	540	600	10	24	8	8	104
80 Flour 20 Potato	25.0	550	687	10	23	7	7	102
70 Flour 30 Potato	42.8	520	743	9	24.5	6	6.5	88
60 Flour 40 Potato	66.6	460	767	7	25	5.5	6	89
50 Flour 50 Potato	100.0	372	744	3	22	3	3	68

NOTE: *a = measured loaf volume on 100 gm. material; b = loaf volume calculated on 100 gm. of flour.*

Effect of Potato on Gas Production and Retention of Fermenting Doughs

In view of the remarkable effect of cooked potato upon loaf volume, it was thought advisable to investigate the action of the vegetable on the gas-producing and gas-retaining power of fermenting doughs. A modification of the method suggested by Bailey and Johnson (1), and subsequently used by Johnson and Bailey (8), St. John and Bailey (12), St. John and Hatch (13), and Karacsonyi and Bailey (9), was employed. The procedure in the present

instance differed from the original method chiefly in that dough from 25 gm. of flour was taken for each determination and 100-cc. beakers were used inside of pint Mason jars to hold the dough, instead of glass cylinders perforated near the top and fitted tightly against the lid of the jar. The apparatus used was designed by Dr. R. K. Larmour, Chemistry Department, University of Saskatchewan, and proved very convenient for the purposes of the present investigation.

Three flours were included in this study, No. 2, 5 and a baker's patent milled from Western Canada wheat containing 13% of protein. The first run was made with flour No. 5 using doughs made with the simple formula alone, as a control, and with the basic plus 5, 10 and 20% of cooked potato. Two controls were run, one to measure the total amount of gas evolved from the fermenting dough, and the second to determine the volume of the dough itself. The other tests were made in duplicate, the dough as mixed being divided immediately after mixing into four equal parts. In this way two readings were obtained for each determination. In all, 14 determinations were made in each run.

TABLE IV

INCREASE IN VOLUME OF DOUGH, VOLUME OF CARBON DIOXIDE LOST FROM DOUGH, AND SUM OF THESE VOLUMES DETERMINED AT 30-MIN. INTERVALS FOR THREE HOURS

Controls			Control + 5% potato			Control + 10% potato			Control + 20% potato		
Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough	Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough	Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough	Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough
13	13	0	12	12	0	12	12	0	12	12	0
67	62	5	57	50	7	58	56	2	51	56	−5
126	83	46	106	77	29	109	78	31	113	80	33
179	91	88	153	83	70	162	90	72	163	95	68
223	99	124	205	93	112	207	99	108	227	100	127
274	102	172	250	98	142	266	101	165	285	107	178
312	107	205	288	101	187	313	104	209	347	112	235

The first run was made over a period of three hours after mixing, readings being taken at ten-minute intervals. The results obtained were quite disappointing, as shown in Table IV. No definite increase in gas evolved or volume of the fermenting dough was evident when the treated doughs were compared with the controls. When baking a series of doughs both with and without potato it had been noted that the difference in size and rate of fermentation became most marked after panning, and increased as the doughs neared the oven. It accordingly seemed pertinent to make the measurements relating to gas formation after the usual three-hour fermentation period. The tests were made, therefore, after the two punches and at the time the doughs would ordinarily be panned. Decided differences between the various doughs were revealed by this method, as shown by the data in Table V.

TABLE V

INCREASE IN VOLUME OF DOUGH, VOLUME OF CARBON DIOXIDE LOST FROM DOUGH AND SUM OF THESE VOLUMES AS DETERMINED AT 30-MIN. INTERVALS FOR TWO HOURS, FOLLOWING THREE-HOUR FERMENTATION PERIOD

Controls			Control + 5% potato			Control + 10% potato			Control + 20% potato		
Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough	Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough	Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough	Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough
Flour No. 2											
19	14	5	22	18	4	19	26	−7	26	22	4
58	43	15	62	53	9	76	60	16	80	73	7
97	70	27	109	86	23	135	100	35	143	117	26
132	85	47	149	102	45	186	118	68	205	130	75
Flour No. 15											
12	14	−2	14	15	−1	17	15	2	12	11	1
52	52	0	64	63	1	74	70	4	84	81	3
89	82	7	117	110	7	134	111	23	161	133	28
122	106	16	166	125	41	190	123	67	230	170	60
Flour No. 5											
12	16	−4	19	17	2	18	13	5	11	11	0
56	47	9	57	50	7	61	52	9	66	60	6
90	74	16	96	81	15	113	92	21	129	110	19
122	96	26	133	101	32	156	122	34	186	139	47

Control + 30% potato		
Increase in volume of dough + CO_2 lost from dough	Increase in volume of dough	CO_2 lost from dough
17	15	2
87	80	7
163	132	31
244	153	91

Fig. 1 shows graphically the total gas evolved, the volume of the dough and the gas lost, over a 120-minute period. Flour No. 5 was used, with potato concentrations of 0, 5, 10, 20 and 30%. A progressive increase in gas evolved is shown in going from 0 to 30% potato, corresponding to a similar increase in dough volume. More gas is lost from the dough with increased dosage of potato, but this loss is more than compensated for by the increased gas production due to stimulation by the potato.

Fig. 2, 3 and 4 represent the data similarly obtained on the three flours.

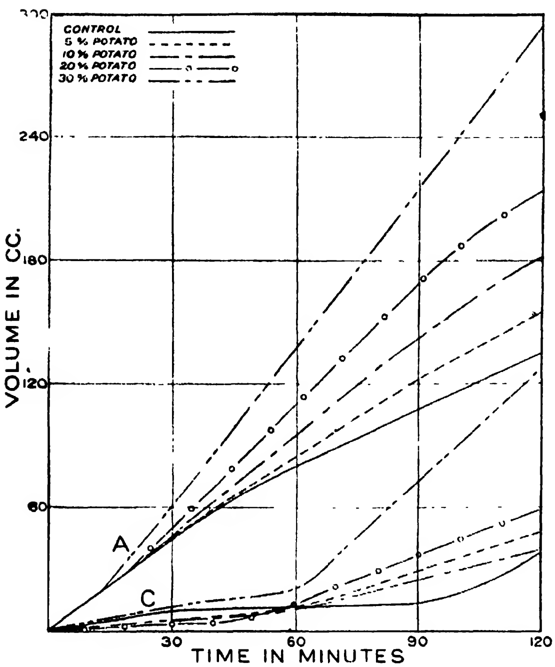

FIG. 1. *Changes in volume which occur in systems containing a fermenting flour dough, and fermenting dough plus potato. Curves A represent the sum of the increase in volume of the dough and the volume of carbon dioxide lost from the dough; Curves C, the volume of carbon dioxide lost from the dough.*

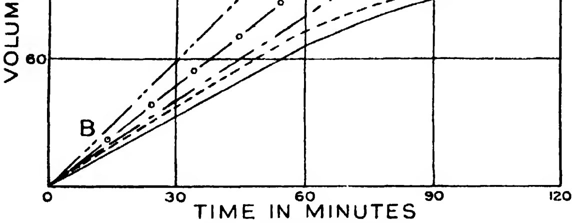

FIG. 2. *Changes in volume which occur in systems containing a fermenting flour dough, and fermenting dough plus potato. Curves B represent the increase in volume of the dough.*

Graph No. 2 shows the total gas evolved, No. 3 the volume of the fermenting dough, and No. 4 the gas lost from the doughs. The principal effect of the potato appears to be an enhanced evolution of gas. This effect increases with increasing potato concentration. The loss of gas also increases with the higher concentrations, but remains less than the increase in production. Flour No. 2 was weaker than flour No. 15, but evolved more gas when not treated with potato. The increase in volume of the dough was less, however, due to inability to retain the gas. It is probable that the soluble nitrogenous constituents of the potato, as well as the dextrinized starch, form a favorable nutrient medium for increased yeast propagation. This effect becomes more marked as the fermentation progresses, hence the doughs containing potato steadily show improvement over the untreated doughs during the later stages of fermentation and in the first few moments in the oven. During the first period, the potato material is probably not readily available for the yeast to act upon, but must be more thoroughly dissolved in the water present in the dough.

Comparison of Effect of Potato, and Malt and Bromate, on Flours of Varying Protein Content

It was considered advisable to ascertain the effect of various percentages of the prepared potato upon a series of flours embracing a wide protein range, comparing the results obtained with the values yielded from

the same series with the use of malt and bromate. Accordingly, a series of 10 flours, as already described, was baked by the simple basic formula and by the basic method plus 5, 20, and 40 gm. of cooked potato added to 100 gm. of flour. A further baking was also made using 50 gm. each of potato and flour. The improver formula as described was also included in a baking series.

The protein content of the flours and the loaf volumes obtained with the various bakings are shown in Table VI. The scores assigned to the loaves are contained in Table VII. Examining first Table VI, it will be noted that the higher protein flours show a surprising reaction to the higher concentrations of the potato, while all the flours exhibit substantial increases in loaf volume. The data would seem to show a general trend toward increasing loaf volume with increasing flour protein in each series of bakings, this tendency being less marked in the case of basic values. A striking similarity is evident between the action of all the doughs treated with potato and the malt and bromate baking. The loaf volumes obtained with the 50-50 blend are remarkably high when calculated on the basis of 100 gm. of flour, and show no evidence of decrease of baking strength with the large proportion of potato to flour.

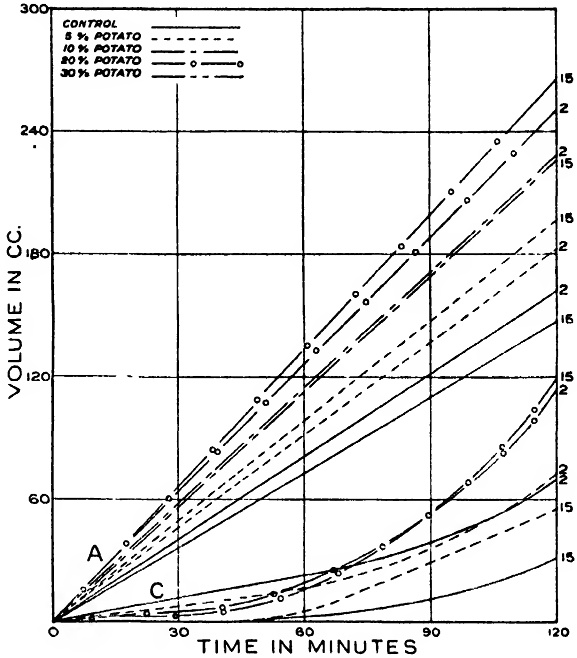

FIG. 3. *Changes in volume which occur in systems containing fermenting flour doughs and fermenting doughs plus potato. Curves A represent the sum of the increase in volume of the dough and the volume of carbon dioxide lost from the dough; Curves C, the volume of carbon dioxide lost from the dough.*

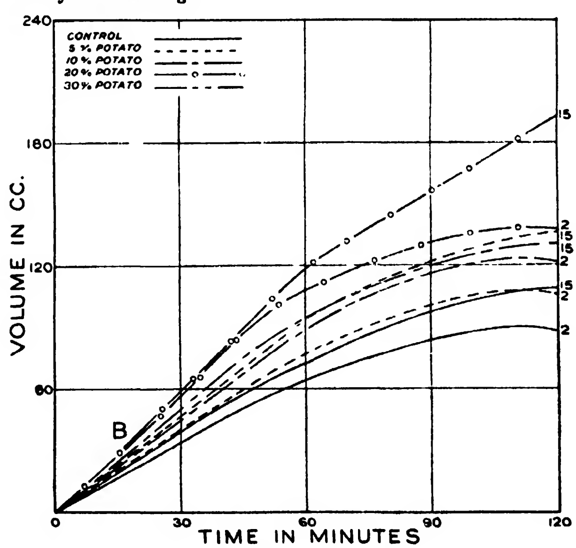

FIG. 4. *Changes in volume which occur in systems containing fermenting flour doughs and fermenting doughs plus potato. Curves B represent the increase in volume of the dough.*

TABLE VI

COMPARATIVE LOAF VOLUMES OBTAINED WITH BASIC FORMULA, WITH BASIC PLUS VARIOUS PERCENTAGES OF COOKED MASHED POTATO AND WITH IMPROVER FORMULA

No.	Protein %	Loaf volume, cc.					
		Concentration of potato, %				Flour, 50 gm.; potato, 50 gm.	Improver method, 1% malt+0.001%$KBrO_3$
		0	5	20	40		
1	7.8	370	380	420	475	420	410
2	10.7	450	450	560	610	590	535
3	11.3	420	478	542	640	570	465
4	11.8	490	532	560	665	700	585
5	12.2	480	530	595	640	750	550
6	13.3	500	545	577	690	800	610
7	13.6	475	530	615	660	740	640
8	17.4	510	560	630	720	780	645
9	17 8	610	652	703	840	900	800
10	18.8	520	600	690	825	960	810
Av.		482.5	525.7	589.2	676.5	721.0	605.0

The average loaf volume is highest for a high concentration of potato although the loaves were of very poor appearance and shape, were lacking in bloom and had a rather heavy soggy interior. These loaf volumes were calculated on the basis of 100 gm. of total material and the values obtained are shown in Table IX. It is seen from this table that the volumes tend to increase up to a concentration of 20% potato, falling at 40% to the basic values, as registered by the average results. The loaf volumes obtained with the improver, however, are larger than the corresponding potato results with one exception, flour No. 3 at 20%.

The baking scores as shown in Table VII reveal little change in color or symmetry with increasing concentration of potato. The grain and texture grow progressively poorer due to the loosening up of the loaf by the potato. The baking score is influenced by the loaf volume to such an extent that an increase is evident here with increase of potato. To obviate this difficulty, the scores were recalculated, using loaf volumes corrected to 100 gm. of total material. These results are shown in Table VIII. The largest single score in this table is yielded by the improver data while the largest increase in the potato treatments over the basic values is shown by the 5% concentration. The 40% treatments have the lowest scores, owing to the poor texture and grain of the loaves baked with this concentration of the vegetable.

Correlation constants were calculated between crude protein and the loaf volumes obtained by the different baking tests. These values are shown in Table X, with the points of minimum significance from the table of values at the 5% points, according to the number of pairs (3). These constants are all very significant, and show a decided relationship between protein and baking strength. The basic results would appear to be slightly less related to protein than the other baking data, but the difference is not significant. The cor-

TABLE VII

BAKING SCORES ASSIGNED LOAVES BAKED WITH BASIC FORMULA, WITH BASIC FORMULA PLUS POTATO AND WITH BASIC FORMULA PLUS MALT AND BROMATE

Flour No.	1	2	3	4	5	6	7	8	9	10	Av.
Color of loaf											
B	15	9	11	19	21	17	19	8	13	8	14.0
5%	14.5	10	11.5	21	21 5	18	19	8.5	14	9	14.7
20%	15	8	12	20	20.5	18.5	20	9	14.5	9.5	14.7
40%	14	8.5	13	20	20	17.5	19	7	14	8	15.1
1	12	10	10	18	16	17	19	9	15	11	13.7
Symmetry of loaf											
B	4	7	5	9	7	8.5	7	8	9.5	9	7.4
5%	4.5	7.5	6	10	8.5	9	8.5	8.5	10.5	9	8.2
20%	5	9.5	7	8	9	9.5	9	6.5	10.5	9	7.5
40%	4	7	8	9	9	10	9.5	6.5	10.5	9	4.0
1	3	8	4	9	7	10	10	9	11	11	8.2
Grain of loaf											
B	6	6	9	9	10	8	8	5	9.5	7	7.7
5%	5	7	8.5	8	9	7	8.5	5	6	6	7.0
20%	4	4	5	6	6.5	6.5	7	4.5	8	7	5.8
40%	2	2	4	5 5	4	5	5	5	7.5	2	4.2
1	6	7	6	7	8	6	8 5	5	7	7	6.7
Texture of loaf											
B	4	6.5	9	9	10	8	7.5	6	8	7	7.5
5%	4	7	9	8.5	10	7.5	8	5.5	7	5	7.1
20%	2	5	5 5	8	9	7.5	8.5	4.5	8	4.5	6.2
40%	1	4	4	7.5	5	8	6	4.5	8	4	5.2
1	4	5	7	6	8	5	7	6	6	7	6.1
Score											
B	66	73	76	95	96	91	89	101	101	83	84.5
5%	66	76	83	101	102	96	97	83	103	89	90
20%	68	82	84	98	104	100	106	87	111	99	94
40%	68	82	93	108	102	109	105	95	114	105	98
1	66	93	73	98	94	89	108	93	119	117	95

relations calculated from the bakings with potato are very similar to the values yielded by those with the improver, and would seem to substantiate the view that potato functions in much the same manner as other, more widely known, flour improvers. Crude protein of flour is shown to be as important in relation to loaf volume when potato is included in the baking formula as when other flour stimulators are used.

These results appear to justify the use of cooked potato in various forms in bread making, as it produces larger loaves of improved color. The improve-

TABLE VIII

BAKING SCORES CORRECTED TO 100 GM. OF MATERIAL

No.	Basic method	Concentrations of potato, %			Improver method
		5	20	40	
1	66	64	64	55	66
2	73	74	77	65	93
3	76	80	79	75	73
4	95	98	93	89	98
5	96	99	99	84	94
6	91	93	94	79	89
7	89	94	100	87	108
8	78	81	82	74	93
9	101	100	105	100	119
10	83	86	93	82	117
Av.	85	87	89	79	95

TABLE IX

LOAF VOLUMES CORRECTED TO 100 GM. OF TOTAL MATERIAL

No.	Loaf volume, cc.					
	Basic method	Concentration of potato, %			Flour, 50 gm.; potato, 50 gm.	Improver method
		5	20	40		
1	370	362	382	340	210	410
2	450	428	509	436	295	535
3	420	455	493	457	285	465
4	490	507	509	475	350	585
5	480	505	540	457	375	550
6	500	519	525	493	400	610
7	475	505	560	472	370	640
8	510	533	573	514	390	645
9	610	621	640	600	450	800
10	520	571	627	590	480	810
Av.	482.5	500.6	535.8	483.4		605

TABLE X

CORRELATION CONSTANTS CALCULATED BETWEEN CRUDE FLOUR PROTEIN AND LOAF VOLUMES OBTAINED BY INCLUDING VARIOUS QUANTITIES OF POTATO IN THE BAKING FORMULA

Baking formula	Correlation constant
Basic method	+ .8479
Basic method + 5% Potato	+ .9017
Basic method + 20% Potato	+ .9342
Basic method + 40% Potato	+ .9442
Basic method + 50 gm. flour and 50 gm. potato	+ .9504
Basic method + 1% diastatic malt and 0.001% $KBrO_3$	+ .9335
Value at 5% point	+ .6319

ment in volume seems to extend to a high concentration of potato, but this gain is offset at these proportions by very poor grain and texture of loaf. The color also tends to fall off when the potato content is high. It certainly is not feasible in view of the extensive demand for a close, even-textured loaf, to replace a substantial portion of the flour by potato.

Conclusions and Summary

1. Cooked potato material has a beneficial effect upon doughs. This effect is especially noticeable in regard to loaf volume and color, and increases with increasing concentration of potato material. At high concentrations, the texture and grain of the loaf are adversely affected. The keeping qualities of the bread appear to be improved.

2. The cooked potato appears to function as a stimulant to the gas production, and while more gas is lost from the dough as compared with doughs made without potato, this loss is less than the gain in gas evolved. The net result is therefore a gain in dough volume. These effects increase as more potato is added to the dough. Strong flours are apparently better able to retain the gas produced than are weaker flours.

3. The beneficial effect of potato upon bread appears to be due to the soluble nitrogenous matter and starch contributed. No noticeable effect was produced by raw, uncooked potato or potato ash. Heating the potato had no effect upon the improver action, and as the potato enzymes would be inactivated by this treatment, the action is not due to enzymes.

4. Loaf volume is significantly related to crude flour protein in all the bakings made with the concentrations of potato used in this study. In this particular, potato corresponds to the action of malt and bromate. Flour protein would therefore be an important factor when cooked potato is used to supplant the usual improvers in the baking formula.

Acknowledgment

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References

1. BAILEY, C. H. and JOHNSON, A. H. Cereal Chem. 1: 293-304. 1924.
2. BLISH, M. J. Cereal Chem. 5: 277-287. 1928.
3. FISHER, R. A. Statistical methods for research workers. 3rd ed. Oliver and Boyd, London. 1930.
4. GEDDES, W. F. Can. J. Research, 1: 528-558. 1929.
5. GEDDES, W. F. and GOULDEN, C. H. Cereal Chem. 7: 527-556. 1930.
6. HARRIS, R. H. Cereal Chem. 7: 557-570. 1930.
7. JAGO, W. The technology of breadmaking. Bakers' Helper Co., Chicago. 1921.
8. JOHNSON, A. H. and BAILEY, C. H. Cereal Chem. 2: 95-106. 1925.
9. KARACSONYI, L. P. and BAILEY, C. H. Cereal Chem. 7: 571-587. 1930.
10. LARMOUR, R. K. Cereal Chem. 7: 35-48. 1930.
11. LARMOUR, R. K. and MACLEOD, A. G. Sci. Agr. 9: 477-490. 1929.
12. ST. JOHN, J. L. and BAILEY, C. H. Cereal Chem. 6: 51-59. 1929.
13. ST. JOHN, J. L. and HATCH, M. Cereal Chem. 8: 207-216. 1931.

STUDIES ON BROWNING ROOT ROT OF CEREALS

II. SOME PARASITIC SPECIES OF PYTHIUM AND THEIR RELATION TO THE DISEASE[1]

By T. C. Vanterpool[2] and J. H. L. Truscott[3]

Abstract

Evidence is presented which shows that browning root rot of cereals is caused primarily by species of *Pythium* the most important of which are *P. arrhenomanes* Drechsler var. *canadensis* n. var. and *P. volutum* n. sp. Soil conditions, especially those following summerfallow, and seasonal climatic factors also play a necessary role. Under experimental conditions *Pythium* injury to cereals manifests itself as an embryo rot or as pre-emergence killing of the seedlings, as post-emergence blighting, or as retarded development throughout the life of the plant, due to the impairment of the root system especially during the seedling stage. Both the spring and winter wheats are susceptible.

The isolation and inoculation methods found convenient in the study of the problem are outlined. *P. arrhenomanes* var. *canadensis* is widely distributed over the province, whereas *P. volutum* appears to be more limited in its range. Specific diagnoses of these two species and a discussion of their taxonomy are given. Other less aggressive species of *Pythium* undoubtedly contribute to the disease complex.

Comparative experiments show that *Pythium* injury to wheat may be as severe as that caused by *Ophiobolus graminis* or by *Helminthosporium sativum*. In general, *P. arrhenomanes* var. *canadensis*, the Louisiana sugar-cane *Pythium* and *P. arrhenomanes* are similar in their degree and range of parasitism, whereas *P. volutum* shows marked differences. Experimental evidence obtained under controlled conditions indicates that the damage caused to young wheat plants by *P. arrhenomanes* var. *canadensis* increases with both increasing soil temperatures and soil moistures. No correlation has been found between the hydrogen ion concentration of the soil and the distribution of the disease. Both *P. arrhenomanes* var. *canadensis* and *P. volutum* will grow in nutrient solutions with a lower pH value than that of any prairie soil tested, but optimum growth for both species occurs at neutrality. No conclusive results have as yet been obtained as to the effects of various fertilizers on the disease under artificial conditions.

Introduction

In recent years much attention has been given to the study of the relation of species of *Pythium* to plant diseases and to root rots in particular. Investigations on the association of *Pythium* species with root rot of cereals in Saskatchewan have revealed an interesting mycological and pathological problem in need of immediate attention. In an earlier paper by Vanterpool and Ledingham (25), it was considered from experimental evidence in the greenhouse that certain species of *Pythium* were the primary causal factor in browning root rot of cereals. The present contribution deals with a study of some of these parasitic species, their relationships to the disease, and attempts to elucidate the conditioning or environmental factors which predispose the host plants to attack. The many striking points of similarity between this root rot of cereals and *Pythium* root rot of sugar cane and corn in the United States, Hawaii, the Philippines and elsewhere, have necessitated the inclusion of comparative studies in this paper. Some recent field data on the disease are also included.

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Contribution from the Plant Pathological Laboratory of the University of Saskatchewan, Saskatoon, Canada, with financial assistance from the Saskatchewan Agricultural Research Foundation.
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General Observations

For a general description of browning root-rot disease of cereals reference should be made to the first paper of this series (25), as the following observations are, for the most part, supplementary to facts recorded there.

Field studies made during the past two summers have confirmed previous impressions that the occurrence of the disease, especially on wheat, is very closely related to the soil conditions brought about by the common practice of summerfallowing, as well as to seasonal climatic conditions. Several cases have been brought to our notice in which the yield of the wheat crop following summerfallow was about equal to or less than the yield of the second or stubble wheat crop on the same land. This was attributed to browning root rot in the fallow crop and its absence in the stubble crop. When, moreover, such infested land is again thoroughly fallowed and resown to wheat, browning root rot is very likely to reappear should weather conditions be suitable. Obviously, the parasitic fungi are present in the soil and what is essential for the appearance of the disease is a set of environmental factors acting on the host, on the parasite, or on both. Browning root rot was not very prevalent during the 1930 and 1931 growing seasons, due probably to the extremely dry conditions which prevailed during the spring of both years. However, throughout the north-central, and northeastern parts of the province, where the rainfall was normal or above normal, there were many scattered localities with severe cases of the disease. The root rot is most common on the brown soil of the Regina Clay type and on the black clay-loam soils of the park-land region, but it is occasionally found on soil of light texture, thereby indicating that soil type is not a limiting factor. Cases of the disease have been found on incipient podsol in the Birch Hills area. During 1930, wheat plants affected with browning root rot were received from Manitoba by Dr. P. M. Simmonds of the Dominion Laboratory of Plant Pathology, Saskatoon. In the same year Robertson (18) found the disease attacking wheat and oats in Alberta. These cases constitute the first reports of the disease outside of Saskatchewan.

The strong drying winds of the springs of 1930 and 1931 with their consequent soil drifting and mechanical damage unquestionably interfered with field diagnosis of the disease, as spots which ordinarily would have been bronzed by browning root rot, and the healthy green areas, alike showed the effects of "blowing." In many instances microscopical examination of wheat seedlings from such areas revealed abundant *Pythium* oospores in brown, flaccid root tips, both so characteristic of browning-affected plants. This masking may, in part, have accounted for the reported absence of browning root rot in many localities.

The first symptoms of the root rot were noticed during the first week in June when the wheat seedlings were about five to six weeks old. This agrees with its appearance in former years and suggests that there might be, under certain conditions, a *critical period* in the growth of the seedlings when their resistance to the attack of root parasites is lowered. That the plants frequently recover, although delayed in maturing, is further evidence in support of this supposition.

In one locality where browning is common a summerfallow wheat crop sown two weeks later than usual still contracted the disease, which, of course, further tended to delay maturity and render the crop more liable to rust infection and frost damage.

Cases were again reported where the disease failed to reappear on the wheat in old straw-stack spots after summerfallowing, although adjoining patches were affected. These facts suggest that the crop residue in the stubble crop possibly acts in some way as a soil amendment inhibiting attack from species of *Pythium*, in spots which were diseased in the previous year's fallow crop. The contradictory evidence regarding farmyard manure as an amendment for the trouble may perhaps be explained on the basis of its straw content.

During the spring of 1930 an examination of some sickly winter-wheat plants in the experimental plots at the University revealed the presence of *Pythium* oospores in brown root-tip lesions. The plants recovered, but showed signs of impaired vitality for the remainder of the season. It seems reasonable to suppose from this that *Pythium* may sometimes be a factor in the root-rot complex of winter wheat in many parts of the winter-wheat areas on this continent. Isolations from these winter-wheat plants several weeks later yielded only saprophytic species of *Pythium* or at most only very weak parasites. Rarely are parasitic species obtained when isolations from spring wheat affected with browning root rot are attempted in late July or early August. Therefore, to obtain the correct relationship of various species of *Pythium* to browning, periodic isolations should be made beginning one or two weeks before the first indications of injury appear, and continuing until recovery is apparent. It has been the experience of the authors that the parasitic species are vegetatively active at an early date, the duration of this activity depending on seasonal climatic conditions. Consequently, isolations made after the active parasites have formed their sexual spores will, in the large majority of instances, yield only fungi playing a secondary role. Unless definite information is available on the present and past conditions of the crop, on climatic and soil conditions, and perhaps other factors such as previous crops, mere lists of the fungous flora from diseased roots will be of little significance. R. D. Rands (17) in the United States, and C. W. Carpenter (1) in Hawaii, have had much the same experience with regard to *Pythium* root rot of sugar cane.

Recent Literature

A serious new disease of maize caused by a *Pythium* of the *gracile* group, and quite distinct from the root rot of maize caused by *Pythium arrhenomanes* Drech. in the United States (*cf.* 12) was reported from Italy by Curzi, in 1929 (3). The specific identity of this fungus was not given. From the same country in the following year Petri (16) described a species of *Pythium* as the cause of a disease of the basal portion of wheat plants in early summer. The *Pythium* disease of wheat in Saskatchewan on the contrary is confined almost entirely to the root system and never have lesions been found on the first and second internodes as illustrated in Petri's paper. Petri did not assign

his fungus to a definite species. Roldan (19) in 1930, published a note on the occurrence of *Pythium* root-rot disease of maize and sugar cane in the Philippine Islands. Whether one or two species of *Pythium* are concerned is not clear. Rice is another cereal which has been known for some time to be affected with a *Pythium* root rot in the Dutch East Indies (13). Sideris (21, 22) has recently reported nine species of *Pythium* (of the Nematosporangium group) which were obtained from diseased roots of *Ananas sativus* as being aggressive root parasites of *Triticum vulgare* and *Zea mays*. The experiments were presumably conducted under artificial conditions. The identification of many of these species which are morphologically very similar is unfortunately based on cultural differences on a variety of plant media some of which (and among them the most important, papaya agar) are unobtainable in temperate climates. Some justification may be found for this procedure, but it unquestionably makes it more and more difficult for workers who have obtained one or more of these morphologically similar species to know definitely what forms they are working with. The accurate determination of the identity of a species would entail an enormous amount of comparative culture work, and would have to be undertaken by one or two authorities on the group. The most common Saskatchewan wheat *Pythium* is morphologically very similar to several species described by Sideris, but whether it is identical with any one of them cannot definitely be determined from the descriptions alone.

Methods of Isolation

The usual method of placing necrotic root tissue on poured agar plates was found to be unsatisfactory for the isolation of parasitic species of *Pythium* or of other parasitic Phycomycetes. Fresh material obtained directly from a field showing early symptoms of browning, or from young plants grown in infested soil in the greenhouse, proved to be best for securing the desired forms. The field material was dug up with the aid of a trowel, care being taken to secure a large lump of adhering soil and not to damage the root system unduly; this was then placed in a covered can for conveyance to the laboratory. Young root tips were selected and after being examined for internal mycelium were kept in sterile water for one or two days; they were then placed on non-acidified water agar or cornmeal agar plates. As the mycelium spread out over the agar, hyphal-tip cultures of fungi of the desired type could be secured. Similar methods of isolation are described at some length by Drechsler (6), and in shorter form by Vanterpool and Ledingham (25). Occasionally, it was possible to isolate parasitic species of *Pythium* from browning root tips which had over-wintered under field conditions.

Preliminary Experiments on Parasitism

With the pure cultures thus obtained a very convenient laboratory experiment for separating the parasitic and non-parasitic forms was then conducted. Apparently sterile wheat seedlings, with plumules and primary roots varying from one-half to three centimetres in length and outwardly free from seed-borne organisms, were selected from a moist-chamber allotment and transferred to

sterile 125-cc. Erlenmeyer flasks, each containing a piece of filter paper 4 to 5 cm. in diameter, and 5 cc. of sterile tap water. The flasks were then inoculated in duplicate with two-day-old agar cultures of each of the isolation forms to be tested and placed on the laboratory table. Uninoculated flasks were kept as controls. In the course of one to three days the pathogenic forms produced a stunting and a yellow to dark brown discoloration of the roots with consequent dwarfing of the shoots. These forms, together with any which may have caused a dwarfing without obvious discoloration of the roots, were then further tested for their parasitic tendencies on the various cereals in pot experiments in the greenhouse. Prolonged use of this preliminary flask method has convinced the authors that both aggressively parasitic species and species which later prove to be only weakly parasitic in pots are readily detected in the flasks. It is an accurate preliminary indicator of parasitism as well as being a time saver.

For the greenhouse parasitism tests the various fungi were grown on a sterile, moist, oat-barley mixture for seven to ten days, after which 10 gm. of inoculum to each 6-in. pot was incorporated with the top three inches of steam-sterilized field soil to which about one-sixth sand had been added. To one series of controls, uninoculated oat-barley medium was added in a similar manner; the other series of controls was left untreated. This medium produced no appreciable toxic effects on the control plants under these conditions, which is in accord with the findings of McKinney and Davis (14). Sand-cornmeal inoculum medium was often found suitable for forms which do not grow well on the oat-barley mixture. The various parasitic species of *Pythium* which the authors have studied grow very poorly or not at all on sterile, moist, crushed oat hulls which are recommended by various workers for other root-rot

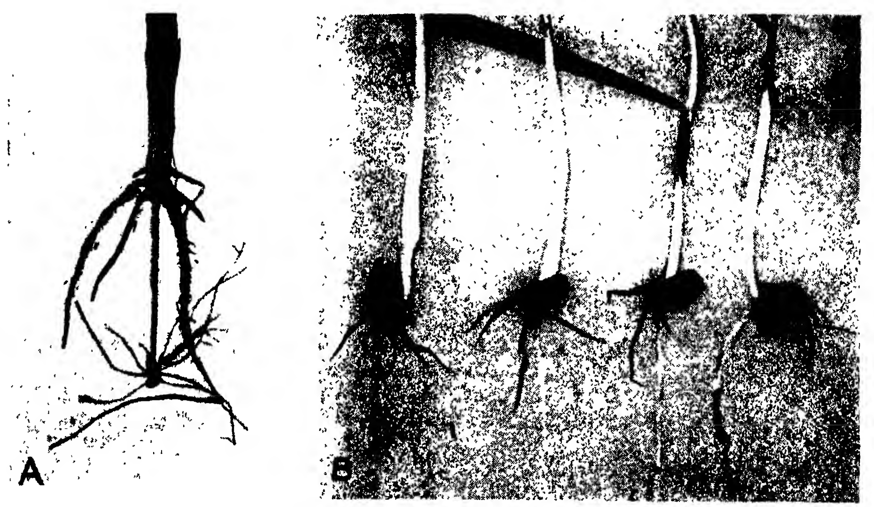


FIG. 1. *A, portion of a wheat plant showing the dark root-tip lesions characteristic of Pythium injury, collected from an infested field. B, wheat plants, four weeks old, grown in pots of steam-sterilized soil artificially inoculated with a pathogenic species of Pythium.*

parasites such as *Helminthosporium sativum*, *Ophiobolus graminis*, or *Fusarium* species. The seed grain was treated with mercuric chloride (1:1000) for 10 min., then washed thoroughly in water, and sown immediately. At the conclusion of the experiments the amount of damage was estimated on the percentage of seedlings in the final stand, their oven-dried weight, their average height, and the conditions of their root systems, and was rated as trace, slight, moderate or severe. The foregoing method was the one generally used in the pot studies reported in this paper. Any departure from this method is given for any specific experiment.

By comparative cultural studies the large number of parasitic forms were grouped into species; a few of the most parasitic strains of each species were then selected for more extensive studies on their relationships to the disease under controlled environmental conditions. By using the above methods it was relatively easy to demonstrate the vigorous parasitism of several species of *Pythium* isolated from decaying wheat roots (Fig. 1, B), but it is a difficult matter to determine their exact relationships to the disease under crop-culture conditions.

Distribution of the Pathogenic Species

As already mentioned, the frequency of isolation of any particular parasitic species in a given season does not necessarily give a true representation of its distribution, because conditions favoring the active growth of these fungi, during which time they yield most readily to isolation, vary considerably in duration over wide areas. However, isolations made from fresh field material, from greenhouse material grown in infested soil, and in a few instances from browning root tips collected from over-wintered stubble material, have yielded vigorously parasitic species of *Pythium* from Alameda, Regina, Moose Jaw, Saskatoon, Vanscoy, Rosetown, Rosthern, Scott, North Battleford, Prince Albert, Prudhomme and Tisdale. The disease is by no means confined to these localities; the list, on the other hand, gives some idea of the wide distribution of the root rot over Saskatchewan. The *Pythium* flora obtained from wheat roots may be divided into three groups as follows:

1. Those forms which are actively parasitic on wheat. In this group are included two species showing vigorous parasitism and at least three species with moderate, though aggressive, parasitic ability. One of these latter species was obtained from wheat roots grown in virgin prairie soil.

2. Those forms weakly parasitic on wheat even under optimum conditions for infection; and,

3. Saprophytic forms.

One or more of the parasitic species in group 1 have been obtained from all of the numerous browning root-rot fields which were given careful study. In the large majority of these fields *Pythium arrhenomanes* var. *canadensis* n. var. appears to be the chief form concerned in the damage, yet in several districts the root-rot situation is complicated by the presence of the other actively parasitic form, *Pythium volutum*, n. sp., and by various other forms, all of which are capable of strong parasitism. In only one locality where browning root rot is

severe have the authors failed to obtain *P. arrhenomanes* var. *canadensis* in their isolations. From this locality, however, *P. volutum* has been readily obtained on many occasions. *P. volutum* and *P. arrhenomanes* var. *canadensis* show approximately the same parasitism on wheat, but *P. volutum* is a much more active oat parasite (Fig. 6 and 7). Its strong parasitism on oats readily separates it from the other species. Although browning root rot can be produced experimentally by at least four species of *Pythium*, in most cases under field conditions, it is probably due to two or more of these species jointly.

Cultural and etiological studies of *P. arrhenomanes* var. *canadensis* and *P. volutum*, the two species considered to be of primary importance in the browning root-rot situation, will be described at some length in this paper.

Pathogenic Species Considered

I. *Pythium arrhenomanes* var. *canadensis* n. var.

No reliable means of identifying the various species of *Pythium* from oospore characters within the cereal host tissue has been discovered. Only rarely have reticulate *Pythium* oospores been observed in wheat roots and it is believed that they are of little or no importance pathologically. It is, however, easy to distinguish all *Pythium* oospores from the resting spores of *Asterocystis radicis*

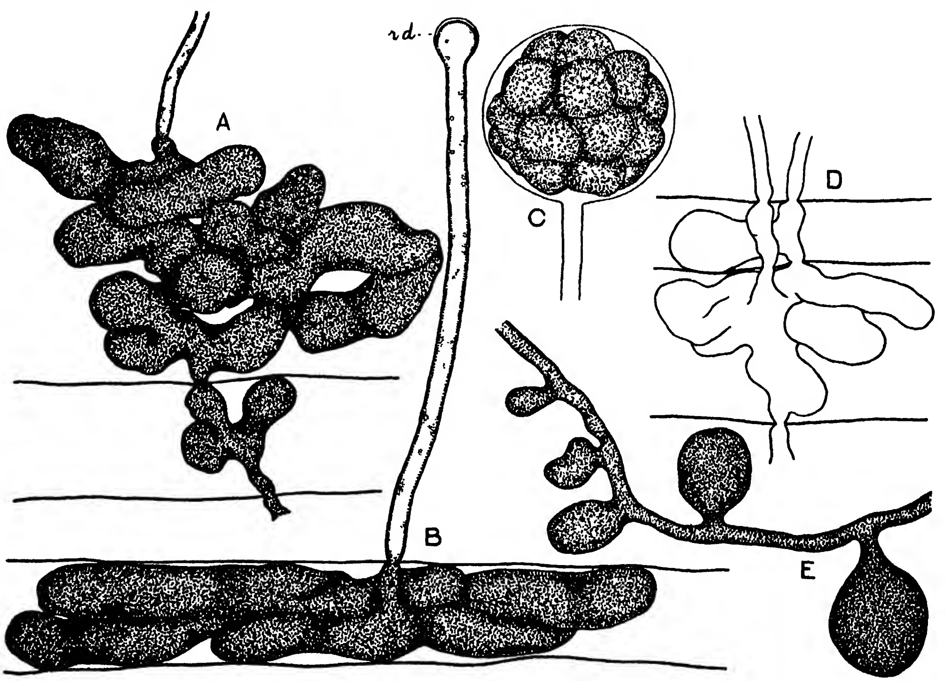


FIG. 2. *Pythium arrhenomanes var. canadensis. A, a lobulate sporangium showing both intra- and extra-matrical development; a portion of the tube of discharge is also shown. B, a sporangium in an epidermal cell of a root immediately before discharging its contents through the evacuation tube into a vesicle at its apex; rd, refractive dome just before being blown out into a vesicle. C, the protoplasmic contents of the vesicle have differentiated into zoospores. D, empty sporangia with portions of their evacuation tubes. E, simple toruloid sporangia. Drawn with the aid of a camera lucida. ×700.*

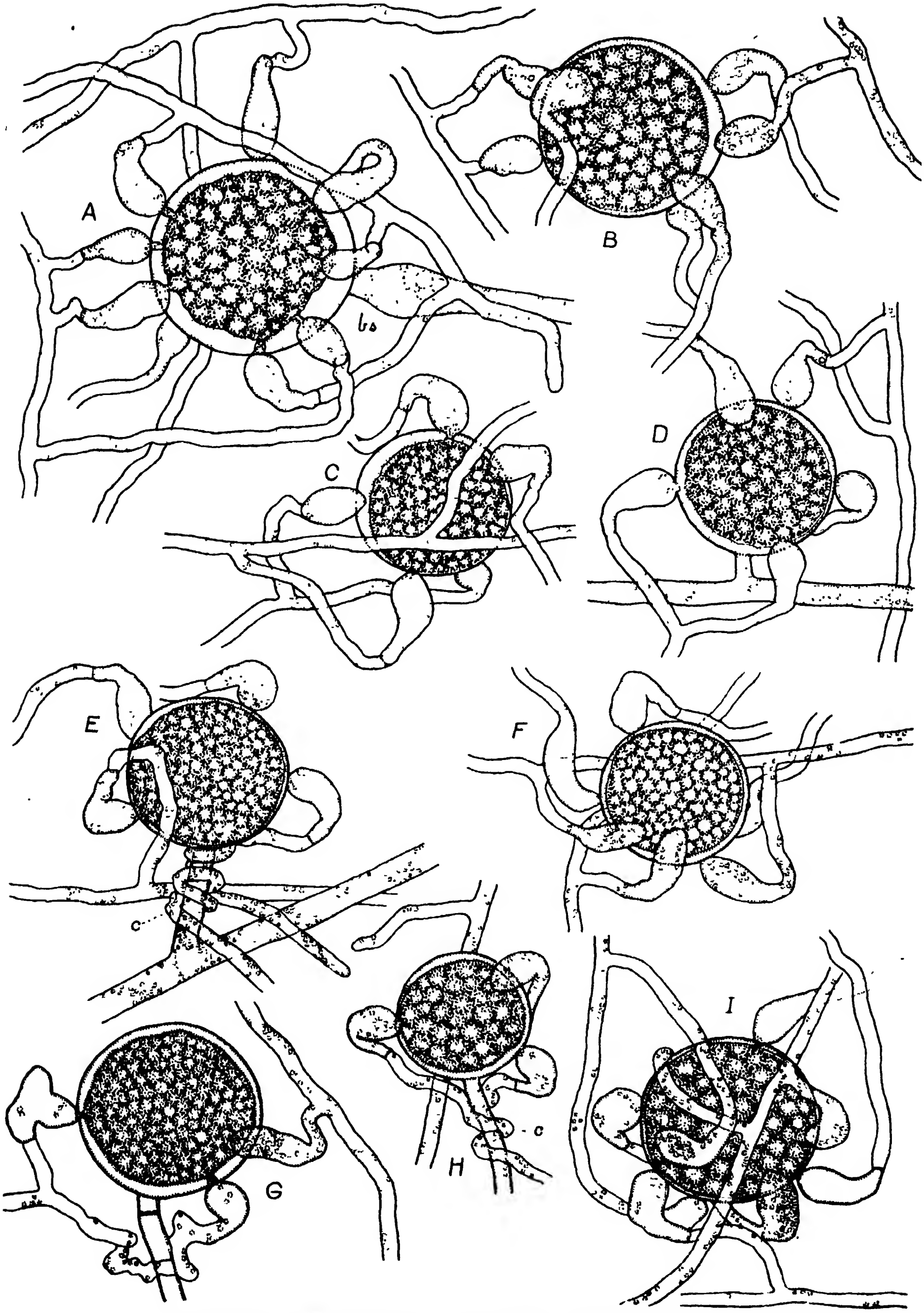


FIG. 3. *A–D, Pythium arrhenomanes var. canadensis, showing the oogonia with the crook-necked antheridia arising from passing hyphae; at A, bs, is a bulbous swelling which is occasionally observed on the oogonial stalk. E–I, Pythium volutum, showing typical oogonia and antheridia; at E, c, and H, c, the antheridial hyphae are shown enlacing the oogonial stalk. Drawn with the aid of a camera lucida. ×700.*

(26). *P. arrhenomanes* var. *canadensis* when growing actively in the host tissues produces lobulate sporangia which at times may fill the whole cell cavity. On this basis it can be distinguished from *P. volutum* which only very rarely produces small toruloid buds and never swollen digitate complexes. Under aquatic conditions the sporangia of *P. arrhenomanes* var. *canadensis* communicate with the outside by means of discharge tubes of which there may be one or more to each lobulate complex, though doubtless the lobulations are separated by septa into compartments, each with its own discharge tube (Fig. 2, A, B, and D). It is difficult to observe these septa before discharge of the sporangial contents. The placing of sporangia-containing roots in water which is changed constantly is usually the best means of initiating zoospore discharge (Fig. 2, B and D). The sporangia, instead of germinating by means of zoospores, often produce one or more germ tubes which develop into ordinary mycelium, in which case they may be said to function as "conidia." Various intermediate abnormal types of sporangial germination have been observed.

In culture, the morphology of the fungus has been studied principally on cornmeal and carrot-cornmeal agars and on steam-sterilized wheat roots in a small quantity of water in 125-cc. Erlenmeyer flasks. It was found that under these cultural conditions there is a greater tendency for the fungus to produce sexual bodies than asexual sporangia, and this holds over a wide range of temperatures. Although oogonia and antheridia are formed abundantly in culture (Fig. 3, A-D and Plate I, 7 and 8), maturation of the oospores occurs only in a small percentage of cases. Instead, the potential oogonia either become emptied of their contents or they may put out several germ tubes (Plate I, 6); this latter phenomenon may happen even after the antheridia have been applied to the oogonia (*cf.* 16). It has been ascertained that oospores formed in wheat roots average 2 to 3 μ smaller in diameter than oospores formed in agar media. Hence the importance of stating specifically the substratum of the oospores of which measurements were taken.

Diagnosis

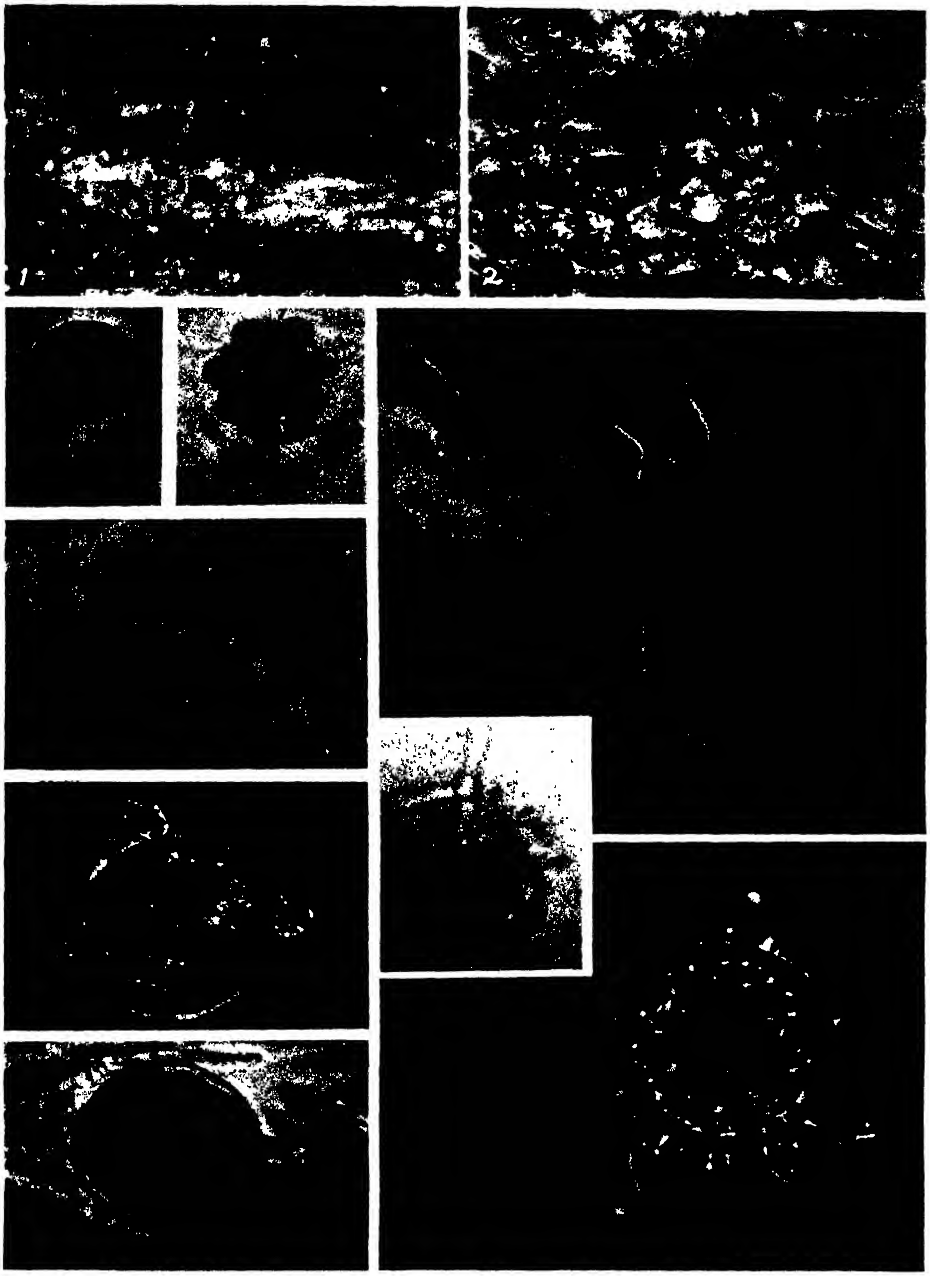
Pythium arrhenomanes var. *canadensis* n. var.

Mycelium finely granular, lustrous and non-septate when young, clear and vacuolate with occasional cross walls when old, branching irregular, aerial development slight, radial growth rate on agar approximately 28 mm. in 24 hr. at 22° C.; optimum temperature for vegetative growth between 30° and 35° C.; lateral falcate outgrowths or appressoria commonly formed in agar plates; in host tissue mainly intracellular, extramatrical development under aquatic conditions.

Sporangia consisting of swollen lobulate elements ranging from toruloid lateral buds to compound complexes, terminal or intercalary, provided with a discharge tube 3 to 5 μ in diameter and up to 150 μ or more in length, seldom formed on solid media but often in liquid media; zoospores from 3 to more than 50 in a vesicle, bi-ciliate, deeply grooved at the hilum region, broad in ventral view, approximately 12 μ when rounded up, monoplanetic, germination by a single germ tube.

Oogonia spherical to subspherical, terminal or rarely intercalary, the

PLATE I



Pythium arrhenomanes var. canadensis. FIG. 1. *Oospores as seen in a typical necrotic root-tip lesion,* ×*150.* FIG. 2. *Lobulate sporangia in the cortex of a wheat root,* ×*600.* FIG. 3. *A vesicle after complete discharge of sporangial contents,* ×*600; stained with lacto-phenol-carbol fuchsin.* FIG. 4. *Zoospores just before discharge,* ×*600; stained with lacto-phenol-carbol fuchsin.* FIG. 5. *Appressoria formed in agar,* ×*200.* FIG. 6. *A potential oogonium which has produced several germ tubes,* ×*400.* FIG. 7. *Oogonia and antheridia on 3-day carrot-cornmeal agar,* ×*600.* FIG. 8. *Same as Fig. 7,* ×*900.* FIG. 9. *An oospore more or less completely filling the oogonium in an artificially inoculated wheat plant,* ×*900.* FIG. 10. *An oospore of unknown species from field material germinating by a germ tube,* ×*600.*

majority ranging from 27 to 40 μ in diameter (average 33 μ) on carrot-cornmeal agar on which they form in two to three days; in liquid culture a bulbous swelling occasionally forms on the oogonial stalk near the oogonium. Antheridia characteristically crook-necked, kneed or clavate, making narrow apical contact with the oogonium, delimited by a single septum, usually 3 to 6 but as many as 12 may be counted, commonly arising from neighboring hyphae, as many as four from one hypha.

Oospores smooth, spherical to subspherical, light brown, usually completely filling the oogonium, average diameter 31 μ, with double wall (2 μ), central globule (17.5 μ), and oblate refringent spot; occasionally oblong oospores with two reserve globules are found.

Cause of a root rot of *Triticum aestivum L.* in Saskatchewan. Also shown to be an aggressive root parasite of *Avena sativa L.*, *Hordeum sativum L.*, *Secale cereale L.*, and *Zea mays L.* Type culture from diseased roots of *Triticum aestivum L.*, Saskatchewan, 1929.

II. *Pythium volutum* n. sp.

Isolations of this species have repeatedly been obtained from the Tisdale district in the park land and the Regina plains in the south. The two strains from these respective regions show consistent minor cultural differences, but these are not considered sufficiently important to warrant their separation into different varieties, though they may be regarded as biologic strains.

On ordinary solid media or agar containing small pieces of grated carrot or wheat roots, no lobulate sporangia have ever been observed. These are rarely ever found in culture; they have been observed on a few occasions in wheat-root-water-culture flasks as lateral outgrowths or buds from the hyphae. Zoospore discharge does not readily occur, only three cases having been observed outside infected wheat roots. In none of these was it possible to distinguish the empty elements within the roots which gave rise to the vesicles, so that the statement that it is the lateral lobulations in this form which function as sporangia is actually based on analogy. On the other hand, typical sphero-sporangia have never been observed. Old agar cultures are dull brown and granular owing mainly to the presence of oospores and numerous empty sterile oogonia. The antheridia arise from neighboring hyphae and commonly coil characteristically around the oogonial stalk (Fig. 3, E and H, Plate II, 1) or, less frequently, around an adjacent hypha. Very rarely, an antheridium has been observed arising from the oogonial stalk. In artificially infected wheat roots in small flasks, oogonia have repeatedly been observed with antheridia in adjoining cells, but applied so that the tip of the antheridium passes through the host cell wall before making direct contact with the oogonium (Plate II, 3). Whether the antheridium forced or dissolved its way through the wall, or whether it passed through a mechanical opening already present, is not known.

Diagnosis

Pythium volutum n. sp.

Mycelium non-septate, lustrous, with an aerial tendency in culture, radial

growth on agar of approximately 16 mm. in 24 hr. at 22° C.; appressoria consisting of lateral falcate structures with rounded ends; mostly intracellular in host tissue.

Sporangia consisting of small lobulations or toruloid buds formed only rarely in aqueous culture; discharge tube usually about 50 μ long and 3-4 μ wide; zoospores biflagellate, bean-shaped, about 10-14 μ.

Oogonia smooth, subspherical, dark brown, terminal on short side stalks or rarely intercalary, formed copiously in culture but a large percentage remain sterile, average diameter 30 μ; antheridia 3 to 10 to each oogonium, crook-necked, sometimes curved or even straight, with narrow apical contact, usually arising from adjacent hyphae each of which supplies one to four antheridia, or more rarely arising from oogonial stalk; antheridial branches commonly entwine about the oogonial stalk in liquid media, but less frequently on solid media.

Oospores smooth, spherical to oblong, usually free within the oogonium, average diameter 27.7 μ, central globule 14.2 μ, refringent spot 8.5 $\times$ 2.2 μ, oospore wall 2.0 μ. Oblong oospores (average 36.9 $\mu \times$ 19.2 μ) with two reserve globules are sometimes present in the host cells.

Causes a root rot of *Triticum aestivum L.* and *Avena sativa L.* in Saskatchewan. Also an aggressive root parasite of *Hordeum sativum L.*, *Secale cereale L.* and *Zea mays L.* when artificially inoculated. Type culture from diseased roots of *Triticum aestivum L.*, Tisdale, Saskatchewan, 1929.

Taxonomic Considerations

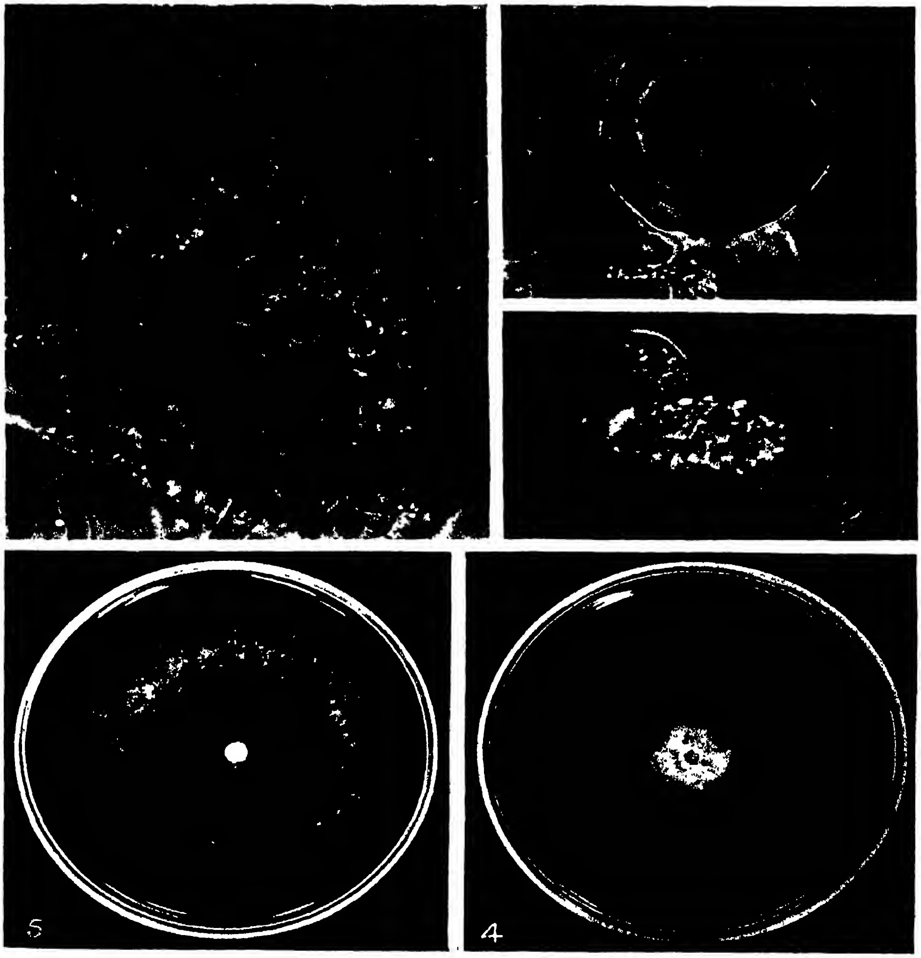
The taxonomy of *P. arrhenomanes* var. *canadensis*, including various isolation strains, deserves some discussion. Because of its lobulate sporangia and numerous crook-necked antheridia, it clearly belongs to the subgenus *Nematosporangium* near to the so-called *arrhenomanes* group. If morphological characters alone are considered, a large number of congeneric isolation strains would readily fall into one species, but culturally and to some extent pathogenically, many morphologically similar strains show quite distinct differences. As such divergent differences may occur in a single strain if this is studied for a long time under a variety of conditions, the correct delimitation of species is by no means a simple matter. Other workers have had similar experiences. Edgerton and coworkers (8), after comparing a United States corn *Pythium* and a Hawaiian cane *Pythium* with their Louisiana cane *Pythium* forms, state that:

"While similar in many ways, these cultures have shown some differences . . . . . It is not yet possible to state whether or not the cultures are distinct enough to represent separate species."

Rands (17) also compared the Hawaiian cane *Pythium* with *P. arrhenomanes* and other congeneric forms. He states that:

"Thus, considering all the tests, there are many points of similarity and of difference in cultural behavior when one attempts to compare any two strains. While they are all obviously very closely related, it remains to be seen whether the differences noted may be constantly correlated with morphological characters or parasitic propensities."

PLATE II



FIGS. 1–4 *Pythium volutum.* FIG. 1. *An oogonium with an antheridial branch coiling about the oogonial stalk,* ×900. FIG. 2. *A mature oospore not filling the oogonial sac,* ×900. FIG. 3. *An oblong oogonium in a root hair, showing an antheridium making contact through the cell wall,* ×900. FIG. 4. *A 2-day culture on carrot-cornmeal agar.* FIG. 5. *P. arrhenomanes var. canadensis, a Petri-plate culture prepared and kept under the same conditions as P. volutum in Fig. 4.*

Sideris (21), however, separates *P. arrhenomanes* and other very closely allied forms mainly on a cultural basis, and states in support of his procedure that, "As morphological differences in the shape and size of oögonia, oöspores antheridia, prosporangia, or zoöspores between species of the same section are almost insignificant, rarely exceeding those of normal variation, the adoption of such characters for differentiation would have been misleading. Physiological and certain morphological differences, however, have been found to be fairly constant as well as stable in certain culture media and for this reason they have been adopted for the differentiation and taxonomic classification of the various species."

After conducting morphological, physiological and pathogenical studies on the Wisconsin corn *Pythium*, the Louisiana cane *Pythium*, and the Saskatchewan cultures, the present authors concluded that the form referred to as *P. arrhenomanes* var. *canadensis* in the present paper, should be regarded as a variety of *P. arrhenomanes* because of its close morphological and pathogenical similarities and its cultural differences with this latter species. This was previous to the publication of Sideris' work. After a study of this paper we do not see that *P. arrhenomanes* var. *canadensis* fits exactly any of Sideris' newly erected species, although it is undoubtedly closely allied to the group which reproduces sexually in one to three days on all culture media. Until there is more general agreement among authorities on the group regarding the methods and characters used in delimiting species of *Pythium*, we feel that it would be best to let the name *P. arrhenomanes* var. *canadensis* stand for the present. The decision that it be regarded as a variety of *P. arrhenomanes* is based mainly on the following differences:

1. Its more ready production of sexual bodies in culture.
2. In general, its less ready production of lobulate sporangia in culture.
3. Its slightly larger oogonia and oospores, and,
4. The presence of 3 to 6 antheridia ordinarily applied to each oogonium. Never more than 12 antheridia have been counted, while in *P. arrhenomanes* they are said probably to double this number.

The comparative parasitism experiments with *P. arrhenomanes*, the Louisiana cane *Pythium* and *P. arrhenomanes* var. *canadensis*, as conducted in the greenhouse and in small field-test plots on cereals and corn, revealed no striking differences, but rather confirmed the close relationship of the three parasites as already indicated morphologically.

The morphological and other characters of *P. volutum*, especially the characteristic coiling of the antheridial branches about the oogonial stalk, are sufficiently distinct from those of any other described species of *Pythium* of the lobulate-sporangium group to indicate that it is a new species.

Comparative studies with *P. aphanidermatum*, *P. butleri*, and *P. graminicolum* have shown that both *P. arrhenomanes* var. *canadensis* and *P. volutum* are distinct from any one of these species.

Recent views regarding the much-needed change in the taxonomy of the genus *Pythium* have been put forward by several writers (7, 10, 20, 23), but we

consider it best for the present to refer our forms to *Pythium* in its broader sense. No attempt will be made in this paper to enter into the discussion on the classification of the genus as a whole.

Pathogenicity

Penetration of the roots of the cereal host by the two species of *Pythium* just described occurs readily through both the epidermal and root-hair cells

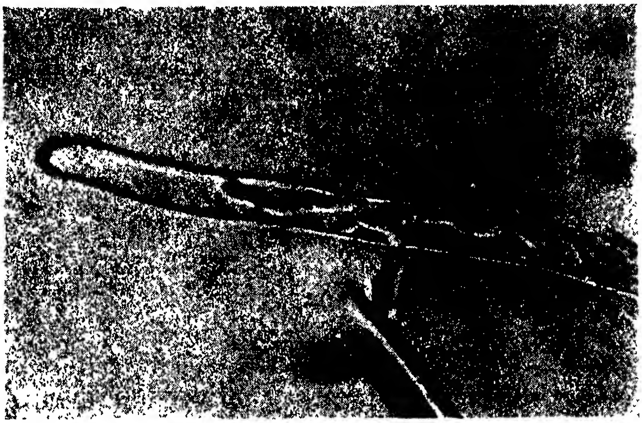


FIG. 4. *A hypha of P. arrhenomanes var. canadensis, penetrating a root-hair cell of a wheat seedling in a water culture. Note the constriction in the hypha at the point of entrance.* $\times 600$.

(Fig. 4). Where the infection hypha comes in contact with the host cell wall, a small appressorium develops and a narrow infection tube pierces the cell wall, and, once inside, regains the normal diameter of the mycelium. The same phenomenon usually occurs when hyphae pass from cell to cell within the host. In so far as can be observed the host offers no resistance to the invading parasite. The growing point of root or rootlet is most commonly attacked, and consequently further growth in length is either retarded or stopped completely. Both fungi grow rapidly through the cortex in all directions and in the course of 24 hr. or less have entered the stele of young roots. Under greenhouse conditions, the affected root tips or girdled portions of the older roots after a time become discolored or necrotic, and present an appearance similar to plants attacked under natural conditions (Fig. 1). It is in these darkened zones that the majority of oospores are found later, although in rare cases of heavy inoculation they may be produced in the coleoptile and the protective sheaths about the crown of the plant. In the pot experiments, oospores do not develop as readily in the discolored root tips as they do under field conditions (Plate I, 1). Browning or yellowing of the outer leaves of seedlings in artificially inoculated soil is occasionally quite characteristic, though ordinarily they remain green even though the root system may be greatly impaired. There is some evidence that sunlight intensity influences these browning symptoms.

It is thus seen that disease symptoms similar to browning root rot in the field can be produced experimentally. Further, re-isolation of the parasites, from the roots of plants in which the disease has been artificially produced, is readily secured.

Comparative Parasitism with other Root-rotting Fungi

An experiment was conducted to ascertain the relative parasitic abilities of *P. arrhenomanes* var. *canadensis* and the well-known foot-rot and root-rot parasites of wheat in this province, namely, *Helminthosporium sativum*, *Ophiobolus graminis*, and *Fusarium culmorum*. The cultures of these last three fungi were the most parasitic Saskatchewan strains obtainable from the Dominion Laboratory of Plant Pathology, Saskatoon, where they have been used in root-rot investigations for some time. All cultures were grown on sterile oat-barley medium in separate flasks and after ten days, 10 gm. of inoculum of each species was placed at seed level in soil contained in 6-in. pots. Twenty grains of wheat were sown to each pot. The soil was kept at a moisture content of approximately 70% of its water-holding capacity. In one series steam-sterilized soil was used and in another it was left unsterilized. The experiment was carried out in duplicate. To one control pot 10 gm. of sterile oat-barley medium was added at seed level.

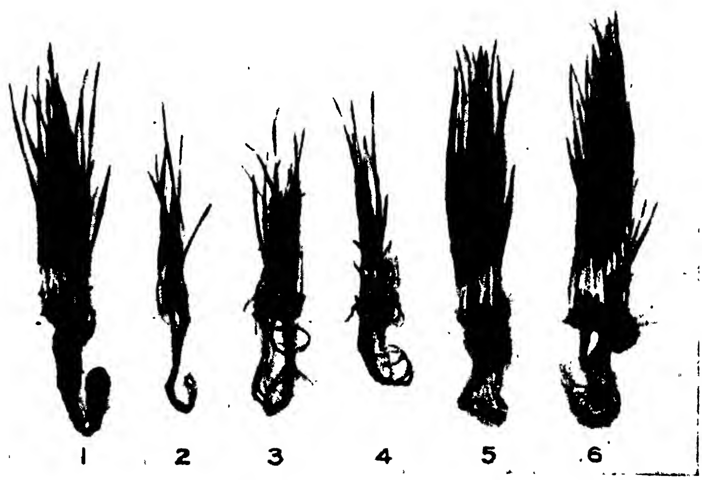


FIG. 5. *Comparative parasitism of various root-rotting fungi four weeks after inoculation. 1, F. culmorum; 2, H. sativum; 3, O. graminis; 4, P. arrhenomanes var. canadensis; 5, Control, plus oat-barley medium; 6, Control, untreated.*

Fig. 5, taken four weeks after the date of seeding, shows the relative damage of the different parasites in steam-sterilized soil during the seedling stage of development. *H. sativum* and *P. arrhenomanes* var. *canadensis* caused about 75% pre-emergence killing. *O. graminis* did not appreciably affect germination, but post-emergence killing of the seedlings reduced the stand considerably. *F. culmorum* did not reduce germination and retarded growth only slightly.

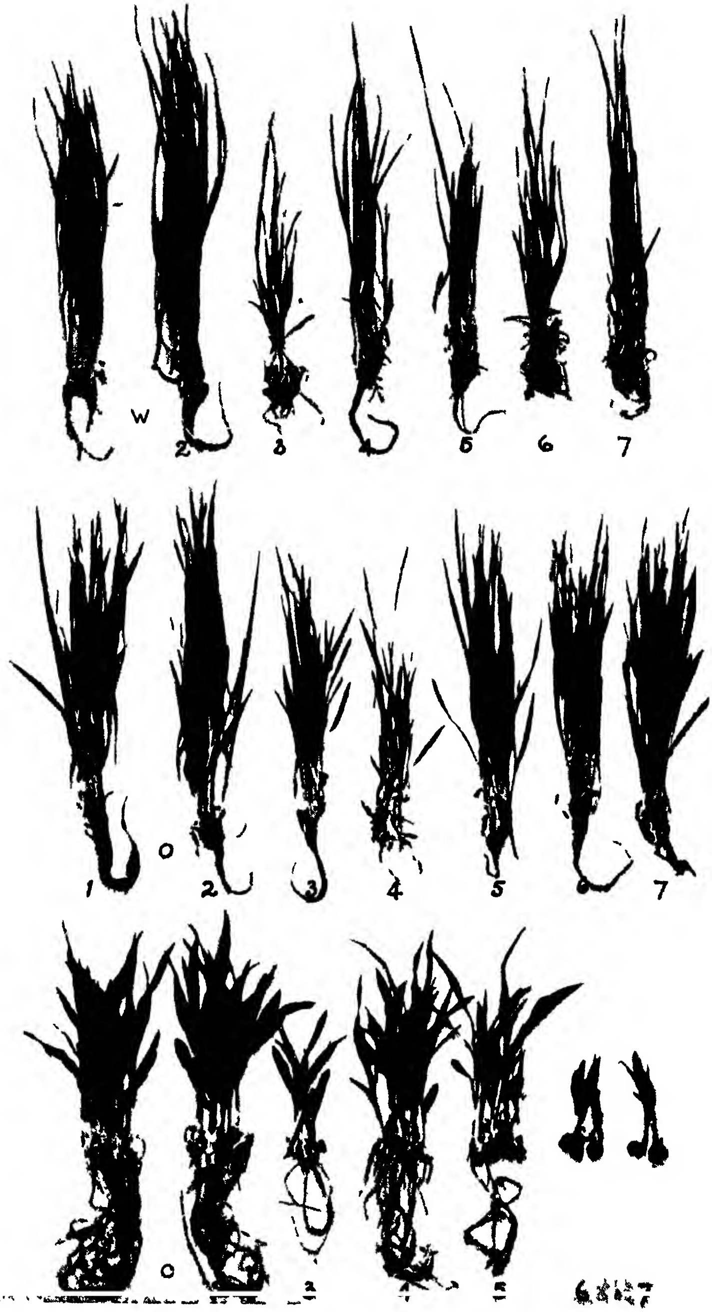


FIG. 6. *Relative pathogenicity of various species of Pythium on whe (W), oats (O), and corn (C), four weeks after inoculation. 1, Control pl oat-barley medium, 2, Control, untreated, 3, P arrhenomanes var canade sis, 4, P. volutum, 5, Louisiana Pythium 931, 6, Louisiana Pythium 143*

Little or no disadvantageous effects were produced on the growth of the seedlings by the oat-barley medium. Damage to about the same order of magnitude was caused by the parasites in the unsterilized soil, but the plants in the control pots of unsterilized soil containing the oat-barley medium were often slightly impaired in their development. In another series of experiments in which the inoculum of each parasite was placed one inch below seed level, *P. arrhenomanes* var. *canadensis* showed the most vigorous parasitism up to four weeks.

The results indicated that *P. arrhenomanes* var. *canadensis* may be as vigorous a seedling parasite of wheat as either *II. sativum* or *O. graminis*, and much more parasitic than *F. culmorum*.

Relative Pathogenicity of Species of Pythium on Cereals

Comparative parasitism experiments between *Pythium arrhenomanes* var. *canadensis* and *P. volutum*, and *P. arrhenomanes* Drech., strain 931 and strain 1432 of the Louisiana sugar-cane *Pythium*, *P. graminicolum* Subram., and a few other congeneric forms, were conducted on various hosts in 6-in. pots in the manner already described for the greenhouse experiments. The temperature varied between 60° and 70° F., and the plants were watered daily. Twenty seeds were sown to each pot except in the case of corn when ten were sown.

Fig. 6 illustrates the plants from a single series of pots in a typical experiment conducted in duplicate, while Table I gives the total number of plants in each pot.

TABLE I.

COMPARATIVE PATHOGENICITY OF SPECIES OF PYTHIUM ON CEREALS

Host plant	Number of plants in each pot after one month						
	Control + oat-barley medium	Control untreated	P. arrheno-manes var. canadensis	P. volutum	La. Pythium 931	La. Pythium 1432	P. arrheno-manes
Wheat (Marquis)	20	20	11	12	14	13	18
Oats (Banner)	20	20	19	14	19	20	20
Barley (Hannchen)	20	20	12	18	16	13	18
Rye (Prolific)	18	19	6	9	9	4	5
Corn (Squaw)	10	10	5	9	8	4	3

The results of this and other similar experiments show the striking similarity in parasitism between *P. arrhenomanes* var. *canadensis* and Louisiana *Pythium* 1432. Louisiana *Pythium* 931 and *P. arrhenomanes* ordinarily give the same results but these do not compare as uniformly with those of *P. arrhenomanes* var. *canadensis* as do those of Louisiana *Pythium* 1432. They are all severely parasitic on wheat, rye and corn and slightly to moderately parasitic on oats

and barley. *P. volutum* differs in its parasitism from all of these other forms. It is severely parasitic on oats, usually equally as parasitic on wheat and rye, moderately parasitic on barley and only slightly parasitic on corn.

P. arrhenomanes var. *canadensis* reduces the stand and arrests the growth of western rye grass and brome grass, on which it may be classed as moderately parasitic. *P. arrhenomanes* and the Louisiana *Pythium* strains were found to be slightly parasitic on these grasses in a single experiment. *P. arrhenomanes* var. *canadensis* causes a trace of damage on flax and peas in pot trials.

Through the courtesy of Dr. C. Drechsler, the authors were able to obtain a culture of *P. graminicolum* Subram. which he obtained from sugar-cane roots. Subramaniam, whose original culture is no longer extant, found the fungus to be the cause of a crown and root-rot disease of wheat in India. Under our conditions of experimentation it is only slightly parasitic on Marquis wheat compared with *P. arrhenomanes* var. *canadensis* and *P. volutum*. *P. butleri* Subram. was not found to be pathogenic on wheat; neither was *P. aphanidermatum* (Edson) Fitzp. (*P. butleri* ?) of the American Type Culture Collection.

The pathogenicity findings substantiate the fact, already arrived at from morphological studies, that *P. arrhenomanes* var. *canadensis* is very closely related to the *Pythium* on corn and the *Pythium* on sugar cane. *P. volutum* is a distinct parasite which is not known to have been described before.

Field Experiments

During 1930 and 1931 various field tests were conducted in small experimental plots artificially inoculated at seed level with oat-barley cultures of the parasites.

In 1930 there were no outstanding differences between the uninoculated rows and rows inoculated with species which proved vigorously parasitic under greenhouse conditions. The inoculated plants did show a few necrotic root-tip lesions, but there was an entire absence of the aggressive parasitism which the greenhouse experiments, in both non-sterile and sterile soil, had led one to expect. The exceptionally dry condition of the soil may have been a factor which rendered the inoculum non-viable before the seed germinated.

The 1931 rod-row results showed definitely that *Pythium* injury to cereals can be obtained from artificial inoculation in the field (Fig. 7). Owing to the dry conditions prevailing, water was applied to the rows at the time of seeding and every two or three days thereafter until the seedlings were about three inches high. Sowings were made on April 30, May 5, May 9, and May 15. The inoculum used was about 7 to 10 days old in each case. The results in the aggregate are in general agreement with those obtained in pot experiments (Fig. 7). Both *P. arrhenomanes* var. *canadensis* and *P. volutum* delayed germination and caused considerable pre-emergence killing. The plants which emerged in the inoculated rows usually grew fairly well, but were always slightly shorter and later than the uninoculated plants. The majority of inoculated plants, however, had varying percentages of necrotic root tips containing *Pythium* oospores. *P. volutum* was more parasitic on both wheat and oats than was *P. arrhenomanes* var. *canadensis*, although they

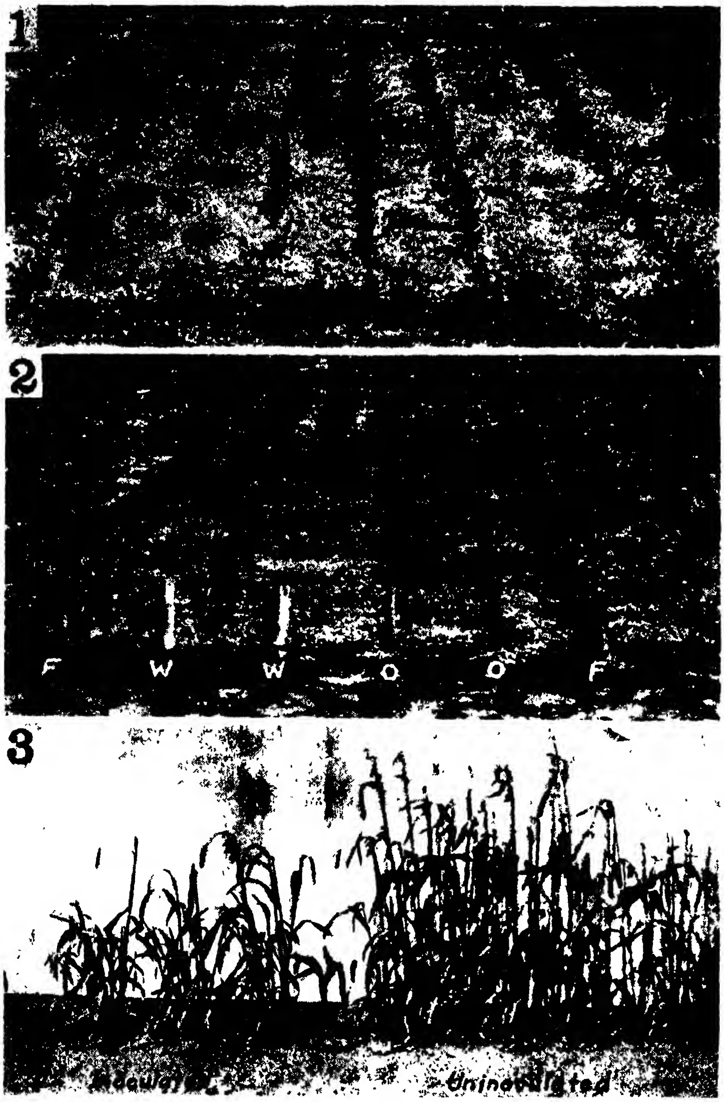


FIG. 7. *The effects of artificial inoculation in the field. In 1 and 2 the front eight feet of each rod row was inoculated, and the remaining eight feet, at the back, was left uninoculated. W, wheat, O, oats; C, corn; and F, filler row of wheat, uninoculated. 1. Inoculated with P. arrhenomanes var. canadensis. 2. Inoculated with P. volutum. 3. P. volutum on oats, showing dwarfing and delayed maturity.*

both caused much more damage to wheat than to oats. Under the field-plot conditions, *P. arrhenomanes*, Louisiana *Pythium* 1432, and the two Saskatchewan forms, are very similar in parasitism on wheat, oats and corn. On the other hand, *P. graminicolum* showed only slight indications of parasitism to wheat under the same conditions. No differences in the effects of various

fertilizers on the disease could be observed in these plot tests.

It was found that the amount of pre-emergence killing was greatly influenced by the state of the inoculum; *i.e.*, whether it had been kept under good growing conditions for 7 to 10 days or whether it had been under adverse conditions or was a little too old. This doubtless interfered with any differences produced by the different dates of seeding.

Too many difficulties attend comparative field experimentation with different species of *Pythium*, and it is felt that a fairly accurate idea of the relative parasitic ability of the various parasites can be obtained from pot experiments in the greenhouse where conditions can be more thoroughly controlled. On the other hand, any clear-cut effects of fertilizers on the disease will probably be ascertained only by conducting experiments in naturally infested fields.

Soil Temperature and Moisture Relationships

To ascertain the influence of soil temperature and moisture on the infection of young wheat plants by *P. arrhenomanes* var. *canadensis*, experiments were conducted in galvanized iron soil cans, 6 in. in diameter and 10 in. deep, in temperature-controlled tanks.

The soil was made up to the required moisture content by weight based on its water-holding capacity. A layer of sterile sand ¼-in. thick was placed on top to prevent the soil from caking or cracking. Surface watering was found to give as good a distribution of moisture as any other method of application. Weighings were made every other day and the necessary amount of water added to maintain the correct moisture percentages. Four temperature tanks containing eight cans each were used. These were maintained at 12°, 17°, 24° and 31° C. respectively, and the air temperature at approximately 17° C.

Inoculations and sowings were carried out in the manner already described for greenhouse pot experiments.

Moisture relations.—From the preliminary temperature-moisture experiments it was clearly shown that the amount of damage to wheat seedlings at all the temperatures increased with increasing moisture content of the inoculated soil.

As a result of this finding, all subsequent temperature-controlled experiments were conducted with a soil moisture content of about 70%.

Temperature relations.—Fig. 8, A, shows plants from a single representative temperature-tank experiment after four weeks. Each bundle represents the final stand from four cans.

The results of this and other temperature-tank experiments indicate that:

(1) The amount of damage to wheat seedlings in inoculated soil, *relative to the controls*, increases directly with increase in temperature. The disease rating was estimated on the percentage of seedlings in the final stand, their oven-dried weight, their average height, and the conditions of their root systems. Actual damage in the inoculated cans is least at 24° C. and increases towards the two lower temperatures as well as towards the higher. It is however consistently worse at 31° C. than at 12° C.

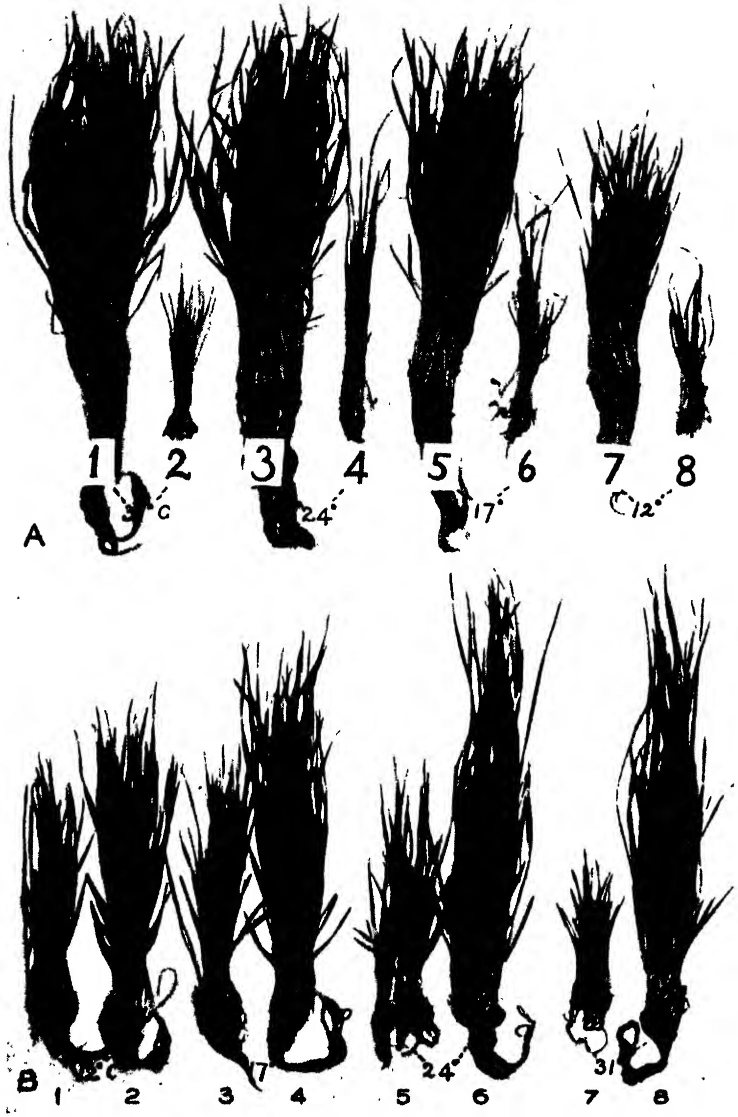


FIG. 8. *The relation of temperature to the disease. A, each bundle represents the final stand after four weeks from eighty seeds inoculated at time of sowing in a single temperature-tank experiment. For each temperature (as indicated in the figure) the bundle on the left represents the control plants and that on the right the inoculated plants. B, for each temperature as indicated, the bundle on the left shows the inoculated plants and those on the right the uninoculated. Inoculation was performed after the seedlings had emerged; both inoculated and controls were then kept at the respective temperatures for two weeks.*

(2) Pre-emergence killing is greatest at the lowest and highest temperatures, although usually slightly worse at the highest.

(3) Post-emergence killing is worst at the highest temperature. The final stand is therefore least at the highest temperature.

Under field conditions the disease symptoms first appear when the seedlings are from 4 to 6 in. high, and, so far as is known, neither pre-emergence killing nor post-emergence killing is common. To circumvent the damage caused in the germination stages in the greenhouse experiments, it was thought necessary to devise some method of inoculating the wheat seedlings after they had reached a height of 1 to 3 in. The following procedure was found well adapted to the particular case in question, and, it seems, might well lend itself to experimentation with other root rots of cereals and other plants. Five disinfected wheat grains were sown in sterilized soil in 2-in. pots, and when the seedlings were 1 to 2 in. high, fifteen seedlings, *i.e.*, the contents of three small pots, were transplanted to a single 6-in. can, care being taken not to damage the root systems or disturb the soil unnecessarily. The cans contained sterilized soil in which 20 gm. of inoculum had been incorporated. Each of the three individual batches of seedlings was so placed that inoculated soil came in contact with the roots on all sides. In the control cans no inoculum was added to the sterilized soil. The cans were then placed in their respective temperature tanks and the soil kept at a moisture content of about 70%.

Fig. 8, B, shows a representative series in an experiment conducted in the manner just described. The seedlings were transplanted on the seventh day and then kept at the respective controlled temperatures for two weeks. The oven-dried weights of these plants showed that the damage at 12° C. was approximately 49%, at 17° C. 51%, at 24° C. 60%, and at 31° C. 76% of the control plants at the respective temperatures.

It seems that *P. arrhenomanes* var. *canadensis* can cause severe damage to young wheat seedlings when inoculation is performed after the plants have emerged, that the amount of damage relative to the controls increases directly with increase in temperature, and that post-emergence killing or seedling blight can occur. These results are in agreement with those obtained when inoculation is done at the same time as sowing.

Hydrogen Ion Relationships

On the disease.—A preliminary study on the relation of the hydrogen ion concentration of the soil on the disease was made by testing the pH of browning root-rot soil and of soil from healthy areas in the same field, and from healthy summerfallow wheat fields during the early growing season. Both the infested and the healthy soils tested were either neutral or slightly alkaline, so that although these conditions may be favorable to the root rot, soil reaction need not be considered of any consequence in the disease situation in the province. Attempts were made in the greenhouse to grow wheat in inoculated soil adjusted to pH values ranging from 3.5 to 9.0. Sulphuric and hydrochloric acids and sodium and potassium hydroxides were used in the soil-reaction adjustments. It was found practically impossible with the prairie soils to maintain an equilibrium at a constant pH on the acid side of neutrality for a sufficiently long time to conduct a worthwhile experiment. This is in all

probability due to the large reserve of carbonates in the prairie soils. In the tests that were made, there were no definite indications to show that acid soils decreased the disease to any appreciable extent.

On the parasites.—Cultural studies have shown that *P. arrhenomanes* var. *canadensis* and *P. volutum* will grow in nutrient solutions with a much lower pH value than that of any prairie soil tested, but that the optimum growth for both species occurs at neutrality. Comparative pH studies have shown the very close affinity between *P. arrhenomanes* var. *canadensis* and the Louisiana *Pythium* 1432 (11) and also suggest that this physiological study may be useful in helping to ascertain the identity of those *Pythium* forms which fail to produce fruit bodies in culture.

A detailed study of the hydrogen ion relationships of *P. arrhenomanes* var. *canadensis* and *P. volutum* will be published in a separate communication.

Fertilizer Experiments

Several fertilizer treatments were conducted under greenhouse conditions on wheat grown in a mixture of browning root-rot soil collected from four localities, in sterile soil inoculated with an oat-barley culture of the parasite, and in field-test plots artificially inoculated. The effects of the following fertilizers were tried, namely, sodium nitrate, ammonium sulphate, potassium sulphate, potassium chloride, ammonium phosphate, triple superphosphate, straw and farmyard manure; especial attention was given to the effects of nitrogenous fertilizers on the disease.

The results failed to show any appreciable differences on the amount of root rot from the various treatments. However, the following general observations may be noted:

1. In the majority of instances, no noteworthy differences in the amount of root lesioning occurred with the various fertilizer amendments. The fertilizer treatments produced various effects on the host, such as differences on growth rate, rather than on the disease.

2. The plants treated with sodium nitrate in a few cases produced discolored coleoptiles and a darker root system than the controls. Usually, however, there were no differences in roots or tops between the nitrate-treated plants and the controls, except that the nitrate-treated plants were greener. Normally, one would have expected an increased growth in tops and roots over the controls.

3. Straw either produced no difference or the plants were better than the controls throughout. This is also contrary to normal expectations.

4. Farmyard manure gave much the same results as straw.

Carpenter (2) contends that *Pythium* root rot of sugar cane in Hawaii is enhanced by nitrogenous decomposition compounds of sugar-cane factory by-products and by excess nitrate; also that bagasse or pure sugar-cane fibre inhibits the disease. Our greenhouse experiments coupled with the knowledge that the nitrate nitrogen is highest when browning root rot is worst, and that on the stubble crop nitrate nitrogen is low when browning root rot is practically absent, suggest that Carpenter's view may hold for browning root rot of wheat

also. This is only suggestive as present results are indefinite and inconclusive; it remains for fertilizer experiments in naturally infested fields to prove or disprove this view.

Seed Treatments

Since the parasitic species of *Pythium* are extensively distributed in the soils of the province and are not seed borne, the commonly practised seed treatments are of no significance in preventing damage from root rot. On the contrary, it has been shown experimentally in the greenhouse that those seed treatments which tend to delay or slightly decrease germination, indirectly increase the amount of a pre-emergence and early seedling damage, possibly because of a slightly weakened condition and because the seedling is exposed to attack from the parasites for a longer time before it can become well established. Greenhouse experiments in inoculated soil indicate that deep seeding with its consequent delayed emergence also reduces germination and increases early seedling damage.

Discussion

It is relatively easy to demonstrate the pathogenicity of species of *Pythium* isolated from the diseased roots of cereal seedlings, but the elucidation of the predisposing factors which operate under field conditions presents more difficulty. The relationship of environmental factors to browning root rot is most important. The amount and intensity of sunshine in the field is believed to have a definite bearing on the expression of disease symptoms, but these factors may be operating only in a limited way when inoculation experiments are conducted in the greenhouse. Thus, in our experiments, root lesioning and injury was usually typical, while the parts above the ground rarely showed the brown discoloration of the outer leaves. The parasitic fungi have been shown to be present in the majority of our wheat-growing soils, so that the immediate problem centres around the edaphic and climatic factors which make the seedling roots, especially on summerfallowed land, susceptible to fungous invasion. It is possible that unbalanced nutrition at this time may play a contributing part. A lag in the growth of the seedlings is brought about in soil of otherwise excellent tilth and with plenty of available moisture. These soil conditions probably favor the rapid spread of the parasitic fungi. The disease is most common in wheat for the simple reason that wheat is the crop almost invariably grown on summerfallowed land.

It is a known fact that under prairie crop culture conditions, the nitrate-nitrogen content of the soil is greatest in May and June in the wheat crop following summerfallow (15, 27, 28), and it is possible that the high nitrogen content of the soil at this time may render the wheat roots susceptible to attack. This contention is supported by evidence in current literature which shows that nitrogenous fertilizers render numerous host plants more susceptible to fungous attack, and especially by the finding of Carpenter (2) that *Pythium* root rot of cane is increased by the application of excess nitrogenous amendments. From numerous fertilizer experiments conducted in pots and in artificially inoculated field plots we have so far been unable to detect any consistent out-

standing effect of nitrogenous fertilizers on the incidence of this disease. The indication is that the direct effect of various fertilizers on the growth of the plants appears to be of greater importance than their effect on the severity of browning root rot. However, the results are in no way conclusive and further fertilizer experiments conducted in naturally infested fields may yield very interesting results.

All evidence indicates that the disease is favored by high soil moisture conditions; also that its severity increases with increase in temperature, but that aggressive parasitism occurs over a wide temperature range providing other conditions are favorable. On the other hand, *Pythium* root rot of corn (12) and of cane, both caused by forms identical or very closely allied to the wheat *Pythium*, are most severe at relatively low temperatures. It appears that here is a case where the optimum temperature for the disease is determined by the particular host plant which the fungus attacks. Wheat is more likely to be adversely affected by higher temperatures and therefore becomes more susceptible at these temperatures while with corn and cane the converse is true. However, the incidence of the disease on wheat and corn under controlled temperatures should be conducted under identical conditions. Such an experiment is contemplated. What appears to the authors to be an analogous instance has already been reported by Dickson (4) for *Gibberella saubinetii* (Mont.) Sacc. attacking corn and wheat.

Under natural field conditions *Pythium* injury rarely manifests itself in reduced germination or as post-emergence dying of the seedlings, but nearly always as a root rot which reduces the size and vigor of the growing plants. That these plants may frequently show marked recovery and mature normally, albeit somewhat later than healthy plants, indicates the lack of production of any toxic substance by the fungous parasite. However, in such cases the injury is expressed in a reduction in yield of three to ten bushels per acre. In pot experiments reduced germination and post-emergence blighting are more common.

Nematodes were found on wheat in one browning root-rot locality, but it is believed that they are of no significance in the disease situation.

A discussion of the taxonomic relations of *P. arrhenomanes* var. *canadensis* and *P. volutum* has already been dealt with earlier in this paper, and will not be considered here.

Finally, it should be pointed out that, from a pathogenic standpoint, the sphero-sporangium forms may be regarded as of little consequence, while the nematosporangium forms, which have been given most attention in these investigations, are of major importance.

Acknowledgments

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References

1. CARPENTER, C. W. Notes on Pythium root rot of sugar cane. I. Hawaiian Planters' Rec. 32: 107-117. 1928.
2. CARPENTER, C. W. Notes on Pythium root rot of sugar cane. VI. Hawaiian Planters' Rec. 34: 83-98. 1930.
3. CURZI, M. A serious new disease of maize. Rend. Accad. Lincei. ser. 6. 10: 306-308. 1929. Abstract in Rev. Applied Mycol. 9: 174-175. 1930.
4. DICKSON, J. G. Influence of soil temperature and moisture on the development of the seedling-blight of wheat and corn caused by Gibberella saubinetii. J. Agr. Research, 23: 837-870. 1923.
5. DRECHSLER, C. Pythium arrhenomanes n. sp., a parasite causing maize root rot. Phytopathology, 18: 873-875. 1928.
6. DRECHSLER, C. The beet water mold and several related root parasites. J. Agr. Research, 38: 309-361. 1929.
7. DRECHSLER, C. Some new species of Pythium. J. Wash. Acad. Sci. 20: 398-418. 1930.
8. EDGERTON, C. W., TIMS, E. C. and MILLS, P. J. Relation of species of Pythium to the root-rot disease of sugar cane. Phytopathology, 19: 549-564. 1929.
9. EDSON, H. A. Rheosporangium aphanidermatus, a new genus and species of fungus parasitic on sugar beets and radishes. J. Agr. Research, 4: 279-291. 1915.
10. FITZPATRICK, H. M. Generic concepts in the Pythiaceae and Blastocladiaceæ. Mycologia, 15: 166-173. 1923.
11. FLOR, H. H. Relation of environmental factors to growth and pathogenicity of Pythium isolated from roots of sugar cane. Phytopathology, 20: 319-328. 1930.
12. JOHANN, H., HOLBERT, J. R. and DICKSON, J. G. A Pythium seedling blight and root rot of dent corn. J. Agr. Research, 37: 443-464. 1928.
13. LEEFMANS, S. Diseases and pests of cultivated crops in the Dutch East Indies in 1929. Mededeel. inst. Plantenziekten. Abstracted in Rev. Appl. Mycol. 10: 298-299. 1931.
14. McKINNEY, H. H. and DAVIS, R. J. Influence of soil temperature and moisture on infection of young wheat plants by Ophiobolus graminis. J. Agr. Research, 31: 827-840. 1925.
15. NEWTON, J. D. Seasonal fluctuations in numbers of micro-organisms and nitrate nitrogen in an Alberta soil. Sci. Agr. 10: 361-368. 1930.
16. PETRI, L. Un'estesa infezione di Pythium su piante di grano. Boll. R. Stazione di Patologia Vegetale, 10: 285-301. 1930.
17. RANDS, R. D. Fungi associated with root rots of sugar cane in the southern United States. Third Conf. Internat. Soc. Sugar Cane Technologists. Java. Bull. 33: 1-13. 1929.
18. ROBERTSON, H. T. The browning root-rot disease in Alberta. Canada Dept. Agr., Rep. of Dominion Botanist for 1930. p. 94-95. 1931.
19. ROLDAN, E. F. The occurrence of Pythium root-rot disease of maize and sugar cane in the Philippine Islands. Philippine Agr. 19: 327. 1930.
20. SIDERIS, C. P. The proper taxonomic classification of certain Pythiaceous organisms. Science, 71: 323-324. 1930.
21. SIDERIS, C. P. Taxonomic studies in the family Pythiaceae. I. Nematosporangium. Mycologia, 23: 252-295. 1931.
22. SIDERIS, C. P. and PAXTON, G. E. Pathological, histological, and symptomatological studies on pineapple root rots. Am. J. Botany, 18: 465-498. 1931.
23. SPARROW, F. K. The classification of Pythium. Science, 73: 41-42. 1931.
24. SUBRAMANIAM, L. S. Root rot and sclerotial diseases of wheat. Agr. Res. Inst. Pusa. Bull. 177: 1-7. 1928.
25. VANTERPOOL, T. C. and LEDINGHAM, G. A. Studies on "browning" root rot of cereals. I. The association of Lagena radicicola n. gen.; n. sp., with root injury of wheat. Can. J. Research, 2: 171-194. 1930.

26. VANTERPOOL, T. C. Asterocystis radicis in the roots of cereals in Saskatchewan. Phytopathology, 20: 677-680. 1930.
27. WYATT, F. A., WARD, A. S. and NEWTON, J. D. Nitrate production under field conditions in soils of Central Alberta (1925-26). Sci. Agr. 7: 377-384. 1927.
28. WYATT, F. A., WARD, A. S. and NEWTON, J. D. Nitrate production under field conditions in soils of Central Alberta. Sci. Agr. 7: 1-24. 1926.

THE SOLUBILITY OF HYDROGEN SULPHIDE IN WATER FROM THE VAPOR PRESSURES OF THE SOLUTIONS[1]

By R. H. Wright[2] and O. Maass[3]

Abstract

The vapor pressures of a number of solutions of H_2S in water have been measured at temperatures between 5° and 60°C. A new type of glass diaphragm manometer having several advantages is described, and a bibliography of flexible glass manometers is given. The results show that Henry's law is not strictly obeyed and that previously reported values may require correction. Discussion of the results is reserved for a later paper.

Introduction

Aqueous solutions of hydrogen sulphide are so widely used for such a variety of purposes that a close examination of their properties is important. In this paper is described a series of measurements designed to give the solubility of hydrogen sulphide in water at a number of temperatures and at pressures other than atmospheric; and leading also to a determination of the degree to which Henry's law is applicable. In a subsequent paper will be given the results of electrical conductivity measurements with similar solutions, determinations of the extent of primary dissociation, and an attempt to explain the results of both papers in terms of kinetic equilibria between molecular species existing in the solution.

Previously recorded data on the solubility of H_2S in water suffer from the incorporation of Henry's law as an implicit assumption, a part of the experimental method. Since the authors have found that the law is not strictly obeyed (although the deviations are not large) the last significant figures of the accepted values (4, 11, 12 p. 259, 18, 21, 22) are probably invalidated at atmospheric pressure, and most certainly so at all other pressures.

The present measurements are based on determinations of the equilibrium pressures of known mixtures of hydrogen sulphide and water confined in an especially designed cell. The results given by this method are more directly applicable than those obtained by the usual gravimetric and volumetric procedures.

The experimental arrangements here described were developed for the purpose in hand, but there are features that may prove useful in other work.

Purification of Materials

The hydrogen sulphide was obtained from a cylinder of the liquefied gas and purified by a process of fractional distillation that has been elsewhere described (23). Laboratory distilled water was used and freed from dissolved gases by repeated freezing and melting *in vacuo*.

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Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada. Constructed from a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry at McGill University.
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Measurement of Vapor Pressures in a Closed System: The Glass Diaphragm Manometer

Using H_2S, it was desirable to employ an all-glass vapor pressure cell to restrict the possibility of stray reactions between the gas and mercury or stopcock grease. None of the hitherto described devices* depending on the flexibility of glass for the indirect measurement of pressure in a closed system appeared to be suitable for this work, and eventually a new variety was evolved combining many of the best features of the previous types (*e.g.*, visibility of the pointer, indifference to temperature, etc.). The instrument is, moreover, fairly easily made.

The various steps in making the device are shown in Fig. 1, and its application to the measurement of vapor pressures in Fig. 3. The sensitive diaphragm was blown at the end of a long tube, ring-sealed into a larger tube closed at the bottom and provided with a side tube for connection to the vapor pressure cell. Affixed to the inside of the diaphragm was a light, thin, glass pointer, long enough to project well above all parts of the cell containing the gas (thus allowing for complete immersion in the thermostat). Vibration of the pointer was damped by filling the tube with light oil. It must be emphasized that to produce a satisfactory diaphragm the form of flattening shown at A, Fig. 1, must be avoided, since the relatively acute angle between bulb and flattened portion results in a very easily broken diaphragm. By making the flattened part smaller, as shown at B, a far thinner yet stronger diaphragm may be made.

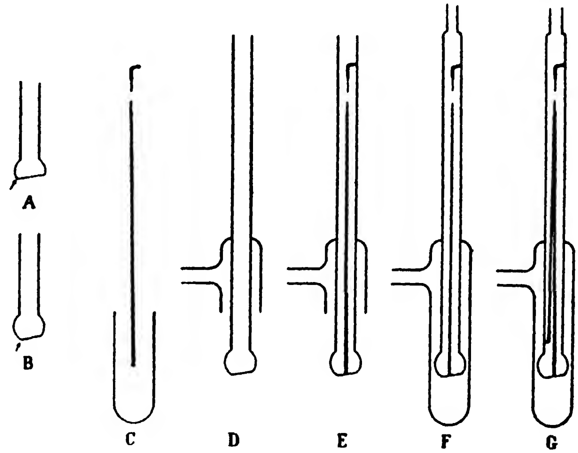

FIG. 1. *Stages in building glass manometer.*

At G, Fig. 1, is shown an alternative type of pointer giving a somewhat greater sensitiveness.

In order to magnify the movements of the pointer, the magic lantern principle was used. By a suitable lens system, enlarged images of the (blackened) tips of the pointer and index (shown in the diagram) were projected on a screen some distance away. Prior to commencing a run, the null position of the pointer with respect to the index (with equal pressures on opposite sides of the diaphragm) was noted. In order to measure unknown pressures thereafter, the external pressure needed only to be made approximately equal to the unknown, for, within a considerable range, the deviation of the pointer from the null point was proportional to the pressure difference producing it, and so,

**A bibliography of flexible glass manometers is given at the end of this paper.*

once the proportionality constant had been found, the internal pressure was quickly obtained. This feature, together with the mechanical advantages of the instrument* and the wide visibility of the pointer image, make the instrument of very great value. In this work, a precision of 0.2% was sufficient, and an instrument reading to 0.5 mm. was easily made. Greater sensitivity could have been obtained if necessary.

Method of Charging the Vapor Pressure Cell

Before determining the vapor pressures of the solutions it was necessary to admit known, and within limits, predetermined amounts of hydrogen

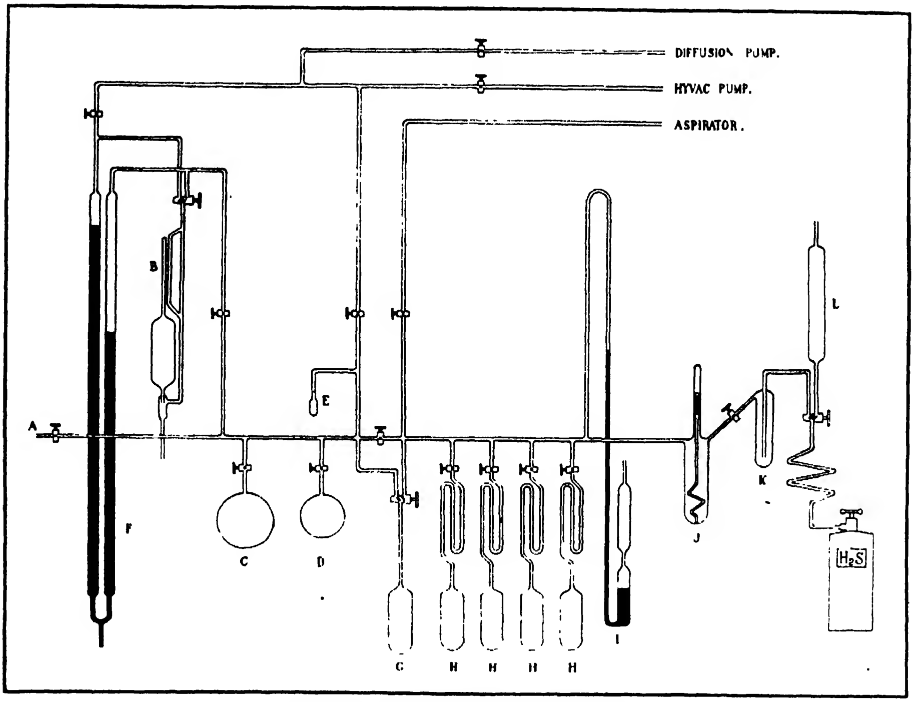

FIG. 2. *Gas measuring apparatus.*

sulphide, into the cell. This was done by the following method. The gas was admitted to a bulb of known volume at a known temperature and pressure and its weight calculated from the vapor density. The tubing was then evacuated and the measured amount of gas condensed in the cell with liquid air. The measuring apparatus is shown in Fig. 2, *G* being the storage bulb for purified hydrogen sulphide, *C* and *D* the calibrated volumes (thermostated at 0° C.), and *F* a manometer reading to 0.1 mm. The vapor densities were determined by us and have been published (23). The estimated precision of the method is 0.1%.

**Note that accidental movements of the lens system or scale, or even of the instrument itself, can be remedied without it becoming necessary to re-determine the null point, a most conspicuous advantage over any other type depending on optical magnification.*

The vapor pressure cell, *M*, is shown in Fig. 3. It contained an electromagnetic stirrer and there was an additional side arm not shown in the figure through which the water was introduced. The U-tube, *N*, was provided to trap any water that might be removed during the extraction of dissolved air as described below. The apparatus of Figs. 2 and 3 was connected at *A*.

Before commencing a run, the cell and attached manometer were removed from the apparatus and the volume found by weighing when filled with distilled water. They were then cleaned and dried and resealed to the apparatus. A known amount of water was run into the cell, *M*, from a weight pipette and the side arm sealed. Dissolved air was thoroughly removed by repeated freezing and melting of the water *in vacuo*.

With the purified water frozen in *M*, a suitable amount of hydrogen sulphide was measured out as described and condensed in *R* with liquid air. The system was sealed off at *X* and allowed to come up to room temperature. Thus, after the commencement of a run the gas came in contact with nothing but Pyrex glass. The cell was arranged so that a water bath could be brought round it. The temperature of the bath was varied between 5° and 60° C. as desired, and hand regulated to 0.1° C. One or two hours were generally

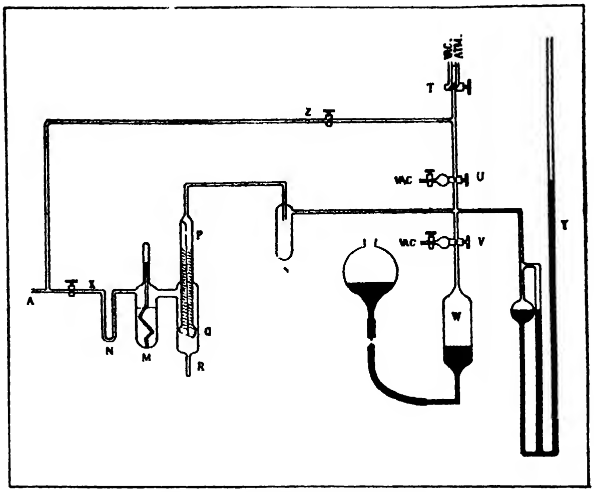

FIG. 3. *Vapor pressure cell.*

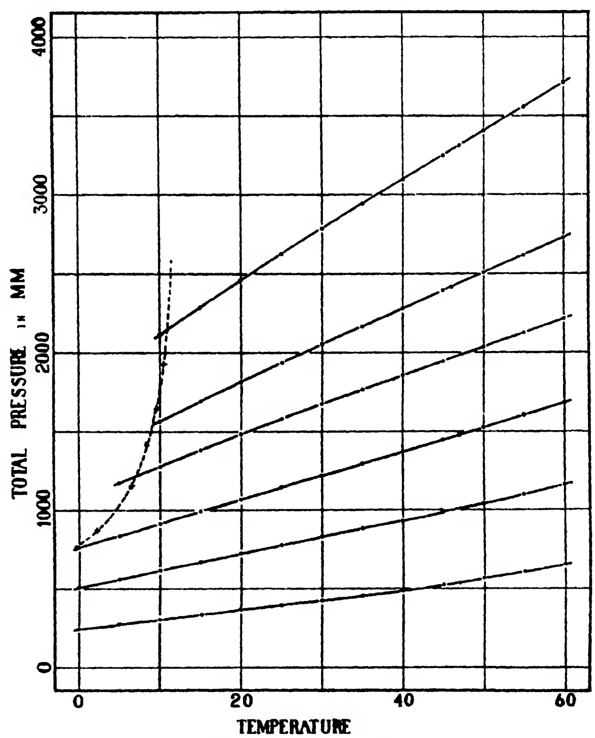

FIG. 4. *Vapor pressure-temperature relation of hydrogen sulphide solutions.*

allowed for the mixture in the cell to come to equilibrium after each temperature change. The method of measuring the vapor pressure has been described.

Experimental Results

The observed pressures were reduced to mm. Hg. at 0° C., and plotted against temperature as in Fig. 4.* (The black circles represent experimental, and the open circles interpolated, values.) Each curve in Fig. 4 represents one run. From the total pressures as observed or interpolated, the internal volume of the cell and the amounts of material in it, the concentration of the solution was calculated at each temperature with the help of the equation:

$$P_1 - P_2 - \frac{WR}{MV} \cdot T + \frac{wR}{MV} \cdot T = 0,$$

where P_1 is the observed total pressure; P_2, the vapor pressure of water at temperature $T°K$; W, the total weight of hydrogen sulphide in the cell; w, the weight of dissolved hydrogen sulphide in the solution; R, the gas constant in cc.-mm.; M, the apparent molecular weight of hydrogen sulphide at temperature T and pressure P_1-P_2; and V, the volume of the vapor phase in cc.

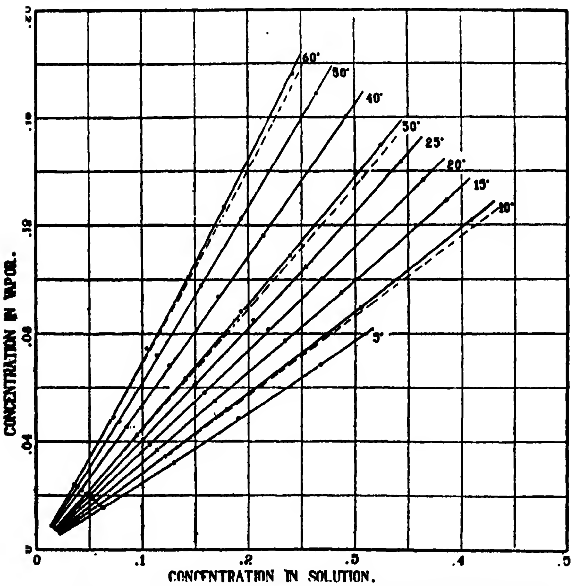

FIG. 5. *Relation between concentration in liquid and concentration in vapor.*

These terms were subject to various corrections. P_1 and P_2 were in mm. of Hg. at 0° C. The apparent molecular weight, M, was calculated from the data of the preceding paper (23).

The volume of the vapor phase, V, was found as follows: assuming the total volume of the cell to be constant over the temperature range covered, the volume of the water was calculated at each temperature and then corrected (by the mixture rule) for the amount of dissolved hydrogen sulphide as obtained from an approximate calculation. The theoretical molecular weights of water and hydrogen sulphide were taken as 18.02 and 34.08 respectively. The equation was solved

**Separation of "H_2S hydrate" from the more concentrated solutions at low temperatures prevented the determination of 5° C. equilibrium points on the higher curves. The position of the phase equilibrium line as determined by de Forcrand (7, 8, 9) is rather indefinite. The dotted line in Fig 4 is plotted from points determined by us:—*

Temp., °C.	*2.2*	*6.6*	*8.4*	*9.7*	*10.5*
Moles H_2S per litre	*0.211*	*0.259*	*0.297*	*0.333*	*0.378*
Total pressure, mm.	*816*	*1190*	*1460*	*1700*	*1960*

for w, the weight of dissolved hydrogen sulphide, and the concentration of the solution then calculated in any desired units.

Table I summarizes the results for each temperature, a cross section of all the results being shown for each temperature. The tables show the total pressure, the partial pressure of hydrogen sulphide (in mm.), concentration of hydrogen sulphide in the vapor and liquid phases (gram-moles per litre),

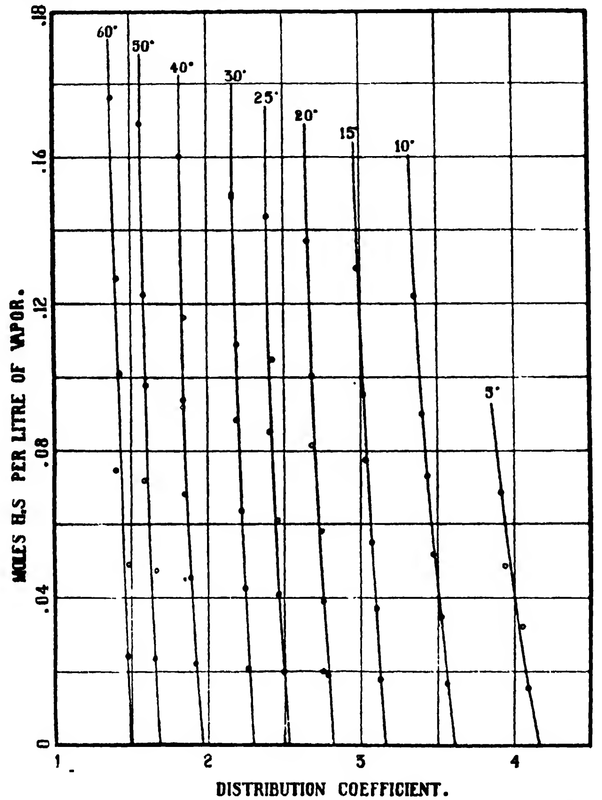

FIG. 6. *Relation between partition coefficient and concentration in the vapor.*

and the partition coefficient, D, of hydrogen sulphide between the two phases. This last has been used instead of the conventional Henry's law constant since it makes due allowance for non-ideality of the gas phase and any variations in it are therefore governed solely by the state of the liquid. For this reason also, in Figs. 5 and 6, concentrations in the liquid and partition coefficients are plotted against the concentration of hydrogen sulphide in the vapor instead of the partial pressure.

Table II summarizes the results and compares them with the values in the International Critical Tables (12). (Table II gives mole fractions divided by partial pressures in mm.) The deviation from Henry's law is evident from the tables and also Fig. 6.

The discussion of these measurements will be given in a subsequent paper.

TABLE I

Experimental Results

Total pressure, in mm.	Partial pressure, in mm.	Moles H_2S per litre of vapor	Moles H_2S per litre of solution	Partition coefficient D	Total pressure, in mm.	Partial pressure, in mm.	Moles H_2S per litre of vapor	Moles H_2S per litre of solution	Partition coefficient D
At 5°C.									
274.5	268.0	0.0155	0.0635	4.09	838	831	0.0484	0.1910	3.94
560	553	0.0321	0.1302	4.06	1176	1169	0.0685	0.2682	3.92
At 10°C.									
303.8	294.7	0.0168	0.0597	3.56	1279	1270	0.0731	0.2511	3.44
615	606	0.0346	0.1220	3.52	1567	1558	0.0900	0.3060	3.40
914	905	0.0518	0.1801	3.47	2112	2103	0.1221	0.4099	3.36
At 15°C.									
333.3	320.6	0.0179	0.0560	3.13	1382	1369	0.0774	0.2346	3.03
670	657	0.0369	0.1144	3.10	1692	1679	0.0953	0.2877	3.02
991	978	0.0551	0.1693	3.08	2284	2271	0.1297	0.3866	2.98
At 20°C.									
362.8	345.4	0.0190	0.0528	2.78	1483	1466	0.0816	0.2188	2.68
724	707	0.0390	0.1074	2.76	1817	1800	0.1005	0.2696	2.68
1067	1050	0.0581	0.1594	2.74	2454	2437	0.1371	0.3642	2.66
At 25°C.									
392.6	369.1	0.0199	0.0497	2.50	1581	1557	0.0851	0.2050	2.41
778	754	0.0409	0.1010	2.47	1935	1911	0.1049	0.2544	2.42
1144	1120	0.0610	0.1499	2.46	2622	2598	0.1437	0.3437	2.39
At 30°C									
422.8	391.3	0.0208	0.0470	2.26	1672	1640	0.0882	0.1932	2.19
830	798	0.0425	0.0955	2.25	2052	2020	0.1091	0.2398	2.20
1219	1187	0.0636	0.1413	2.22	2785	2753	0.1498	0.3247	2.17
At 40°C.									
486.5	431.6	0.0222	0.0426	1.92	1853	1798	0.0937	0.1722	1.84
934	879	0.0454	0.0858	1.89	2278	2223	0.1162	0.2149	1.85
1370	1315	0.0682	0.1260	1.85	3095	3040	0.1603	0.2921	1.82
At 50°C.									
562.4	470.4	0.0235	0.0387	1.65	2033	1941	0.0979	0.1560	1.59
1040	948	0.0474	0.0789	1.66	2505	2413	0.1223	0.1937	1.58
1522	1430	0.0719	0.1139	1.58	3402	3310	0.1690	0.2647	1.57
At 60°C.									
652.2	503.3	0.0243	0.0359	1.48	2213	2064	0.1010	0.1440	1.42
1162	1013	0.0492	0.0730	1.48	2731	2582	0.1269	0.1777	1.40
1681	1532	0.0747	0.1045	1.40	3707	3558	0.1762	0.2424	1.38

TABLE II

SUMMARY AND COMPARISON OF RESULTS WITH THOSE IN THE INTERNATIONAL CRITICAL TABLES

Temp., °C.	$\frac{\text{Mole fraction}}{\text{Partial pressure}} \times 10^6$				Temp., °C.	$\frac{\text{Mole fraction}}{\text{Partial pressure}} \times 10^6$			
	I.C.T. value	1 Atm.	2 Atm.	3 Atm.		I.C.T. value	1 Atm.	2 Atm.	3 Atm.
5	4.185	4.22	4.12	4.04	30	2.161	2.18	2.17	2.16
10	3.594	3.62	3.57	3.54	40	1.767	1.79	1.78	1.77
15	3.111	3.14	3.11	3.10	50	1.488	1.52	1.50	1.49
20	2.727	2.75	2.74	2.72	60	1.279	1.34	1.31	1.30
25	2.416	2.43	2.42	2.41					

References and Bibliography of Flexible Glass Manometers

1. Baume, G. and Robert, M. Comp. rend. 168: 1199-1201. 1919.
2. Bodenstein, M. and Katayama, M. Z. physik. Chem. 69: 29-51. 1910.
3. Bodenstein, M. and Katayama, M. Z. Electrochem. 15: 244-249. 1909.
4. Bunsen, R. Ann. 93: 1-50. 1855.
5. Daniels, F. J. Am. Chem. Soc. 50: 1115-1117. 1928.
6. Daniels, F. and Bright, A.C. J. Am. Chem. Soc. 42: 1131-1141. 1920.
7. de Forcrand. Comp. rend. 94: 967-968. 1882.
8. de Forcrand. Comp. rend. 106: 1402-1405. 1888.
9. de Forcrand. Comp. rend. 135: 959-961. 1902.
10. Gibson, G. E. Proc. Roy. Soc. Edin. 33: 1-8. 1912.
11. Heinrich, F. Z. physik. Chem. 9: 435-443. 1892.
12. International Critical Tables, v.3, McGraw-Hill. 1928.
13. Jackson, C. G. J. Chem. Soc. 99: 1066-1071. 1911.
14. Johnson, F. M. G. Z. physik Chem. 61: 457-463. 1908.
15. Karrer, S., Johnston, E. H. and Wulf, O. R. Ind. Eng. Chem. 14: 1015-1016. 1922.
16. Ladenburg, E. and Lehman, E. Ber. 8: 20. 1906.
17. Preuner, G. and Brockmöller, I. Z. physik Chem. 81: 129-170. 1913.
18. Schoenfeld, F. Ann. 95: 1-23. 1855.
19. Smith, D. F. and Taylor, N. W. J. Am. Chem. Soc. 46: 1393-1396. 1924.
20. Warburg, E., Leithaüser, G. and Johansen, E. Ann. Physik, 24: 25-42. 1907.
21. Winkler, L. W. Z. physik. Chem. 9: 171-175. 1892.
22. Winkler, L. W. Z. physik. Chem. 55: 344-354. 1906.
23. Wright, R. H. and Maass, O. Can. J. Research, 5: 436-441. 1931.

DISPERSION AND SELECTIVE ABSORPTION IN THE PROPAGATION OF ULTRASOUND IN LIQUIDS CONTAINED IN TUBES[1]

Part I

By R. W. Boyle[2], D. K. Froman[3] and G. S. Field[4]

Abstract

An experimental study by the ultrasonic method of the phase velocity of longitudinal waves transmitted in liquids contained in tubes. Greatly augmented as well as largely decreased velocities may be obtained in any liquid by suitably adjusting the frequency of the wave or the diameter of the containing tube. This phenomenon, described here at length for the first time, is found to be caused by the selective absorption of energy of the longitudinal wave at certain frequencies, resulting in a velocity-frequency curve analogous to the "anomalous dispersion" curve of optics. In the experiments there is strong indication that the absorbing frequency depends inversely on the diameter of the tube.

The fact that the absorption frequency does not depend on the material or length of the tube, or for thin walls on the wall thickness, indicates that it is neither longitudinal nor flexural (lateral) vibrations in the tube walls which causes the phenomenon; and the fact that for any liquid the critical frequency shifts with change of diameter indicates that it is in the column of liquid itself that the energy absorption or transference takes place.

These experiments show that it is only at frequencies far removed from absorption, *i.e.*, on the regular and flat portions of the velocity-frequency curve some distance from the discontinuity, that the usual theories of sound transmission may safely be applied.

Introduction

The modern uses of short-length ultrasonic waves have introduced new and convenient methods by which the velocity of sound in solids, liquids and gases may be more completely investigated. New and interesting results by this method on the velocities in liquids contained in tubes prove that it is easily possible to cause at will largely augmented as well as diminished phase velocities; that while the thickness of the tube wall, its elasticity and density may have a certain small importance, these factors are not nearly as effective in causing marked changes in phase propagation in liquids as the frequency of vibration and the diameter of the vibrating liquid column. While lateral waste of energy in the tubular walls and viscous damping in the liquid may be influencing factors on the phase velocity in the liquid column, the dominating factors fixing the velocity are the factors of motional impedance and selective absorption. The experiments here described offer abundant proof of this conclusion, and there are theoretical reasons as well which are discussed in Part II of this paper.

The Helmholtz-Kirckhoff theory was propounded to explain the diminution of the velocity in fluids contained in tubes, which was almost invariably observed by previous experimenters. While some researchers have claimed that this theory sufficiently explained the facts, others were of the opinion that no theory adequately accounted for them all. Most of the experimental

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work in tubes has been performed on gases, generally air, and in this connection Cornish and Eastman (6), whose work lends support to the Helmholtz-Kirckhoff theory, recently summarized much of the previous research on this important question.

Relatively little experimental work has been carried out in liquids as the contained fluid; but it may be recalled that Dörsing reported certain cases of increase of velocity as compared with the velocity in the same liquid when unconfined. Dörsing's (7) method of measurement was that of the Kundt's tube, and, unlike most of the previous experimenters in this subject, he worked at rather high, though audible, frequencies, about 4000 vibrations per sec. These notes were generated by friction, by rubbing metal rods longitudinally with a motor driven friction device. The conclusions Dörsing reported relevant to the experiments of this paper are summarized as follows: "(1) The velocity of sound in liquids contained in tubes, contrary to the case for gases, *increases* with decreasing radius for a given strength of wall, and for a given radius *increases* for increasing wall strength. (2) When the velocity *decreases* in tubes, the decrease may be ascribed almost exclusively to the elastic forces of the tube and of the liquid; heat conduction and friction are without appreciable influence. (3) Vibrating liquid columns generate sympathetic longitudinal vibrations in the tube wall; hence to obtain Kundt's dust figures, it is desirable to make the natural frequencies of the liquid and tube as nearly equal as possible. (4) In vibrating liquids, contrary to the behavior of gases, the harmonic overtones distinctly assert themselves." In these conclusions are found certain important considerations not comprised in the Helmholtz-Kirckhoff or any similar theory. Busse (5) reverted to Dörsing's method to determine velocities of sound in many liquids for the purpose of determining certain useful thermodynamical constants.

On theoretical considerations there should be corrections applied to the unconfined velocity in a fluid for the waste of energy laterally in the vibrations of the wall. If the wall is not rigid, a damping of the vibrations in the contained fluid is caused by the wall vibration, which damping causes a diminution of phase velocity in the contained fluid. This diminution, however, is usually small.

This phenomenon was predicted by Helmholtz in 1846 and first observed by Wertheim in 1847. Since then the problem has been investigated mathematically by Lamb (11, 12), Green (8), and others, and most recently by Gronwall (9). The last undertakes an exact solution of the problem to find the relation between the velocity of sound C in the column of liquid in the pipe or tube and the velocity C_0 in an unlimited body of the liquid. As might be expected the relation is extremely complicated, but by employing suitable approximations the equation may be reduced to $C_0 = \frac{C}{1-E}$, where $C = 2Ln$, L being the length of a liquid column under fundamental resonance, and n the resonant frequency of the generating note; E is a complicated function of the inner and outer radii of the tube wall, the chief elastic constants of its material, and the density of the liquid. Pooler (13) found verification of Gronwall's relation

experimentally by determining the velocities in a column of liquid contained in a vertical cylindrical steel tube. The column was brought into resonance *at an audio frequency* by an electromagnetically excited diaphragm at the bottom. When the resonance frequency of the liquid column was the same as that of the diaphragm the reaction of the diaphragm on the system was very small, and the velocity under this condition was easily measured.

Virtually all velocity of sound measurements in liquids contained in tubes, with those of Dörsing, Busse, and Hubbard and Loomis (10) as notable exceptions, have been made at low, *i.e.*, audio frequencies, and consequently in tubes whose diameters were very small compared with the length of the wave. It is this fact more than any other which has heretofore masked the effect of selective absorption which by the use of ultrasonics may be easily disclosed. Some of the results of the present paper support Dörsing's observations, but they offer a different explanation for a few of his observed facts.

General Method

The object of the researches here described was to study by the ultrasonic method the phase velocities in cylindrical tubes when the propagating medium is a liquid. It was first intended to use tubes of material in which the specific acoustic resistance (ρV), where ρ is the density and V the velocity of sound in the material, was less than, or comparable with, that of the liquid. In such tubes, the walls could not be considered "rigid" in the theoretical sense. But in the progress of the work it soon became evident that this consideration in comparison with others later described was not of great importance, and it became more convenient to use tubes more nearly "rigid" than at first intended. The frequencies employed in the experiments ranged from about 10,000 to 200,000 cycles per sec., and the phase velocities were measured by the method of stationary waves produced by reflection from a reflector in the tube, or, as in later experiments, by identical oscillators facing one another from opposite ends of the tube.

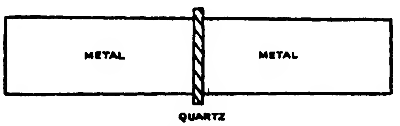

FIG. 1. *Metal rod oscillator.*

The source of ultrasound in the experiments was a simple metal rod oscillator (Fig. 1), or two oscillators, set into longitudinal vibration by the piezoelectric action of a thin plate of quartz appropriately cut. The simple types of oscillators as used in these experiments have often been described, as well as their method of operation by a high frequency electrical oscillation circuit. The metal rods used in the oscillators were generally duralumin, with diameters slightly less than the internal diameters of the experimental tubes. Their lengths were sufficient to permit the convenience of employing the same oscillator without change of experimental arrangement at the first few overtone frequencies as well as the fundamental. The extreme end of the oscillator was fitted snugly inside the experimental tube at one end, the rest of the oscillator being free from the tube but supported at its middle in a thin wooden stirrup. Leaking of the experimental liquid between the oscillator and wall of the tube was prevented by a light packing of soft rubber or suitable

wax. The earliest experiments, of an approximate and preliminary nature, in 1928, were made with a single oscillator and a reflector to create the stationary waves (2).

It is well known that if the acoustic resistances differ greatly for two media the reflection coefficient for sound travelling in the one medium and incident on the other is high. Consequently, as water was the first liquid used, it was decided to employ an air reflector to create the stationary waves, for the acoustic resistances of air and water differ very greatly. The air reflector, Fig. 2, consisted of a flat, thin, sheet of mica M, fitted over the mouth of a bell-shaped piece of metal at the end of an open tube TT. The bell was of such a size that it fitted snugly into the experimental tube. The electric power applied to the oscillating circuit was rectified 60-cycle a.c. which generated in the oscillating circuit a "tonic train" of ultrasonic waves. Though the high frequencies employed were usually ultra-audible in pitch, the 120-cycle note of this tonic train could easily be distinguished by means of a stethoscope attached to the end of the listening tube TT projecting from the reflector.

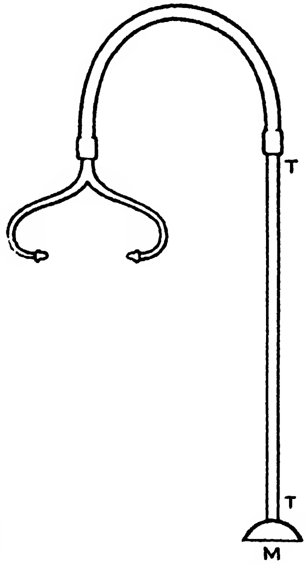

FIG. 2. *Air reflector, shown attached to stethoscope.*

Measurements of the wave-lengths were made by adjusting the position of the reflector in the experimental tube until the sound of the tonic train was a maximum. In this condition the column of liquid between the oscillator and reflector was in resonant vibration, a node of velocity occurring at the mica sheet. The position of the reflector with respect to a measuring scale was noted and then adjusted to the next maximum, which was, of course, one-half wave-length distant from the first. This process was carried out throughout the whole length of the tube, or in the case of extra long tubes, until the distance from the source of the waves became so great that the points of detectable maximum amplitude became indistinct. The velocity of the ultrasound in the liquid was determined by measuring the frequency of the generating electrical oscillations with a Hertzian wave-meter and calculating the velocity from the simple wave relation $v = n\lambda$.

It was at first intended to employ tubes of small rigidity; consequently the first trials were carried out with tubes of sheet celluloid, 0.04 cm. thick, made by rolling the sheets into a cylindrical form of single thickness and cementing together the overlapping ends. A few tubes of mica, made from mica sheets, were also used, but this material broke too easily when rolled into tubes of small diameter. In the first trials the experimental tube was set up vertically, the ultrasonic oscillator being held at the bottom end.

At very high frequencies the nodes and antinodes of the stationary waves were found to be very distinct, and the measured phase velocity was about the same as the unconfined velocity in a large body of the liquid. A small reduc-

tion in velocity might have been possible, but it was soon realized that with a wave-length short in comparison with the diameter of the tube, the velocity in the column of liquid was the same as in an unconfined volume. As the wave-length increased however, *i.e.*, as the frequency diminished, the phase velocity as measured by the stationary waves *was found to increase markedly, and the ease with which the nodes and antinodes could be distinguished became decidedly less.* The readings taken in a typical case are given in Table I, and a curve of velocity against frequency is plotted in Fig. 3. At frequencies lower than those quoted, standing waves could not be detected in tubes of this diameter, *viz.*, 2 cm. Several curves similar to this one were obtained.

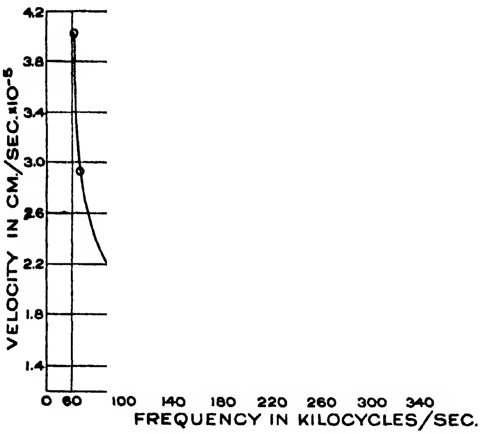

FIG. 3. *Curve of phase velocity against frequency. Water in tube made of celluloid sheet; internal diameter of tube, 2.0 cm.; wall thickness, about 0.4 mm.; temp., 18°C.*

TABLE I

EXPERIMENTAL PHASE VELOCITIES FOR A NUMBER OF FREQUENCIES

No. of antinodes observed	Frequency in cycles/sec.	Wave-length in cm.	Velocity in cm./sec.	No. of antinodes observed	Frequency in cycles/sec.	Wave-length in cm.	Velocity in cm./sec.
31	306,000	0.496	1.52×10^5	11	99,500	2.03	2.02×10^5
21	178,000	0.888	1.58×10^5	9	66,000	4.44	2.93×10^5
10	111,300	1.71	1.90×10^5	4	61,500	6.65	4.02×10^5

NOTE:— *Tube: Celluloid sheet; internal diameter, 2.0 cm. Wall thickness, about 0.04 cm. Liquid used—water. Temperature, 18° C.*

Results similar to the above were obtained on changing slightly the details of the experiment, though not its principle. For example, rubber and other materials were employed as walls of the experimental tubes, or the tubes were made more accurately cylindrical, or other "listening" detection devices were employed, such as a hollow steel reflector having a small central hole covered with a thin mica disk. Slight irregularities of wall thickness or of sectional shape made no great difference. The velocity-frequency curve was always of the same type as that of Fig. 3, within the range of detectable stationary waves. But it was observed that as the internal diameters of the experimental tubes were increased, though the velocity-frequency curves retained the form of Fig. 3, the high values of velocity were shifted towards the lower frequencies.

Further experiment soon showed it possible to detect stationary waves at frequencies lower than those corresponding to the higher velocities, for any

particular diameter of liquid column. For example, in the case of a celluloid ("pyralin") tube with water as the liquid, the velocity-frequency curve was as shown in Fig. 4.

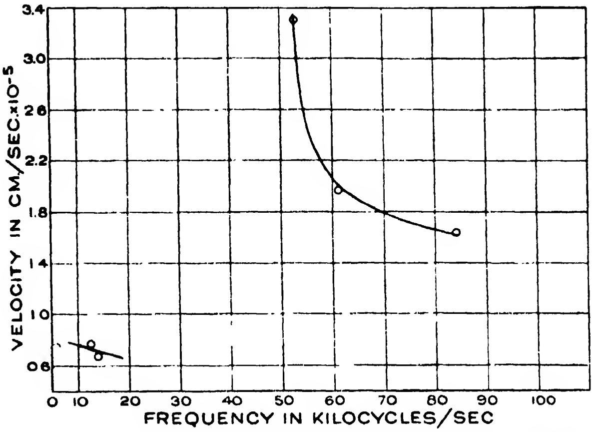

FIG. 4. *Curve of phase velocities for a number of frequencies showing both low and high values of velocity. Water in a pyralin tube, of internal diameter, 2.0 cm.; wall thickness, 1.5 mm.; temp., 18°C.*

The important points to notice about the curve in Fig. 4 are: (1) near the high velocity values *on the lower frequency side* there is a gap in the curve which could not be filled in by any observations depending on the detection of stationary waves; (2) at the frequencies below this region where stationary waves again existed, the velocities were markedly low; (3) the curve is similar in type to the selective absorption ("anomalous dispersion") curve of optics. Other curves similar to Fig. 4 were obtained, but in spite of many experiments it was not found possible by observations to map the curve more completely. Consequently other and better methods of experiment were devised.

Method I

An experimental tube of glass of internal diameter 3.5 cm., lengths varying from 10 to 30 cm., was set up horizontally, and a rod oscillator fitted into one end. The other extremity of the tube projected through a hole in one end of a small tank of dimensions 60 by 8 by 6 cm. (width), and a reflector of metal was placed in the tank at some distance from the mouth of the intruding tube. The tank and the tube were filled with water charged with cinder dust. A diagram of the arrangement is shown in Fig. 5. When the ultrasonic oscillator was operated the cinders formed stationary dust figures (3) in the glass tube, and on sifting more dust into the water in the tank, figures of stationary waves were easily disclosed in front of the reflector. Measurements of the velocity in the tube and in the tank were taken in this way, and compared with those taken by the listener method. The resulting velocity-frequency curves for the tube were the same from both dust figure and listener methods, and similar to the one shown in Fig. 4. However it is important to note that whereas, in the tank, the stationary wave dust figures were formed and the velocity was found to be the same at all

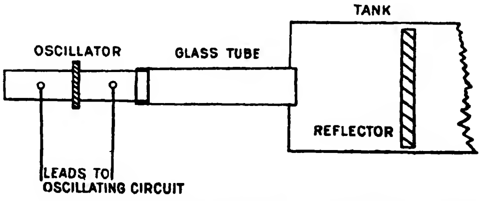

FIG. 5. *Dust figure method for showing confined and unconfined phase velocities.*

frequencies, in the tube the dust figures would not form at those frequencies at which stationary waves could not be detected in the tube by the former "listening" method. At these frequencies stationary waves in the tube apparently did not exist.

Incidental

A special narrow tank was constructed with a rectangular cross section and with ends and bottom of wood and sides of celluloid. The dimensions were: length of tank, 70 cm.; height, 8 cm.; and width, 3 cm. The end of a rod oscillator, of 1.9 cm. diameter, intruded through a hole in one end of the tank, and a movable metal reflector to form stationary waves could be placed in the tank at any desired position. The liquid in the tank was water. By means of both dust figure and listening methods the stationary wave field was surveyed. The resulting velocity-frequency curve was the same by both methods and was very much like that of Fig. 4. In this case the cross section of the liquid column was not circular, nor was the liquid completely surrounded with a wall, which showed that the great variation of velocity with frequency was independent of the sectional form of the liquid column, and of its partial or complete enclosure by a wall.

Method II

In experimental methods based on stationary waves it is often advantageous *to see* the representations of nodes and antinodes from which the measurements are taken. Consequently in the further course of this research it was decided to use tubes with *transparent* walls and to take advantage of the phenomenon of *ultrasonic cavitation* to make clear the position of the nodes and antinodes in the tube. In this method bubbles of gas produced in the liquid by the ultrasonic energy form curtains marking the nodes of the stationaries, and one can easily see when the wave-lengths are shortened or lengthened. There is here also an advantage over the dust figure method in that the present method avoids the necessity of adding dust or other impurities to the liquid.

If, at any given pressure in a liquid containing dissolved gas, stationary waves exist and are sufficiently energetic, small bubbles of gas form throughout the liquid and are driven to the nodes of displacement by the pressure of radiation of the waves. If an ultrasonic beam be directed vertically upward through the liquid column, layers of these bubbles will be formed, one-half wave-length apart, parallel to a horizontal reflecting surface; if the beam and column are horizontal the bubbles will be driven to the nodes and rise in vertical curtains, one-half wave-length apart, in the nodal planes (1, 4).

Naphtha is a liquid easily made to bubble (4) by cavitation, consequently it was now employed as an experimental liquid, enclosed in either glass or pyralin tubes. The tubes were set up vertically at first, the length of the naphtha column being adjustable. Stationaries were produced by reflection at the free air-liquid surface, and the wave-lengths measured by the distance between parallel layers of bubbles (1, 4). The results of a typical experiment are plotted in Fig. 6. It will be noticed that the gap shown in previous velocity-frequency curves is now more completely filled, but it was observed

that only *a very few regular nodes* could be detected at frequencies near those of the minimum or maximum velocities of the curve. Any formation of stationaries in this frequency range was very poor; the nodes were few and also irregular.

The curve of Fig. 6 bears a resemblance to the well-known selective absorption ("anomalous dispersion") curve of optics where the index of refraction (which is proportional to the reciprocal of the velocity) is plotted on a wave-length base. Although in Fig. 6 velocity has been plotted against frequency, this results in the same form of curve as is obtained by plotting reciprocal of velocity against reciprocal of frequency (which is proportional to wave-length). Hence the two curves are comparable, and the characteristic fall, sharp rise and further fall are unmistakable. Selective dispersion is caused by the selective absorption of energy at the frequency of the sharp discontinuity of the velocity-frequency (or reciprocal of velocity and frequency) curve. It thus appears that in the present case there is a special absorption or transference of energy at and near the frequency of the maximum in the velocity-frequency curve of Fig. 6. Under such conditions of absorption it would be difficult or impossible for stationary waves to form in the tube.

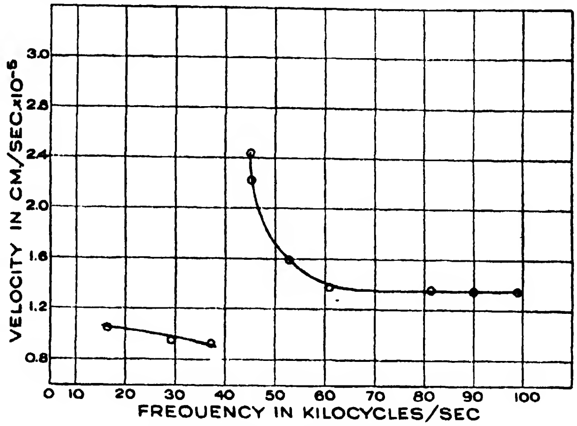

FIG. 6. *Complete curve of phase velocities for a number of frequencies; naphtha in a glass tube.*

Thus the problem of this research developed into an investigation to determine how the energy was absorbed at the "absorption frequency" and how this frequency depended on the dimensions of the column of liquid, the dimensions of the tube walls, and on the materials of both. For this purpose an improved method of experiment was devised.

Method III

By using two transmitters to produce the stationary waves, one at each end of the experimental tube, instead of relying on the reflection by a single reflector at one end only, it was found that more perfect stationaries could be produced, especially at the troublesome frequencies of stationary wave measurement near the minimum and maximum of the velocity-frequency curve. Identical transmitters, connected *in parallel* to the oscillating electrical circuit, were fitted into the opposite ends of the experimental tube (2). This tube was provided with a delivery tube of small diameter near the middle, through which the experimental tube could be filled with the experimental liquid, and to which a vacuum pump could be attached to facilitate cavitation in the liquid

by lessening the internal pressure. The arrangement is shown in Fig. 7. The distance between the transmitters could be varied slightly, but this had little effect on the stationary waves unless the tube was so long that there could be a significant loss of ultrasonic energy intensity between the tube ends and its centre.

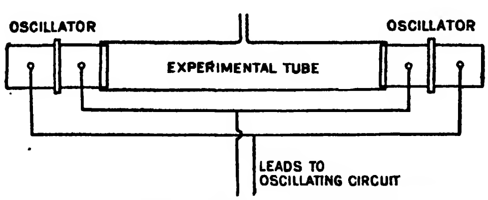

FIG. 7. *Final experimental arrangement.*

That this method of experiment was a great improvement on the former methods could be seen in the fact that more and better-formed stationary waves could be detected at the difficult frequencies in the vicinity of the maximum and minimum of the velocity-frequency curve.

Various Liquids

The position of the break in the velocity-frequency curve, in other words' the absorption frequency, for any given diameter of the experimental tube would be expected to depend on the contained liquid, and, for any selected liquid, to depend on the diameter of the tube.

Experiments illustrating these points were carried out on water, naphtha, castor oil, transformer oil, and chloroform, under the same experimental conditions and consecutively in the same experimental tube. As examples, results for naphtha and transformer oil are shown graphically in Fig. 8. Changes in the energy intensity of the ultrasound emitted from the oscillators made no appreciable difference in the results: on occasions the high frequency voltage applied to the oscillators was doubled, thereby increasing the ultrasonic energy intensity about fourfold, but without any noticeable effects on the velocity-frequency curves. Transformer oil was found to be a very convenient liquid in experiments such as these, for the bubbles produced in it by cavitation were finer and rose through the liquid more slowly, owing to its greater viscosity.

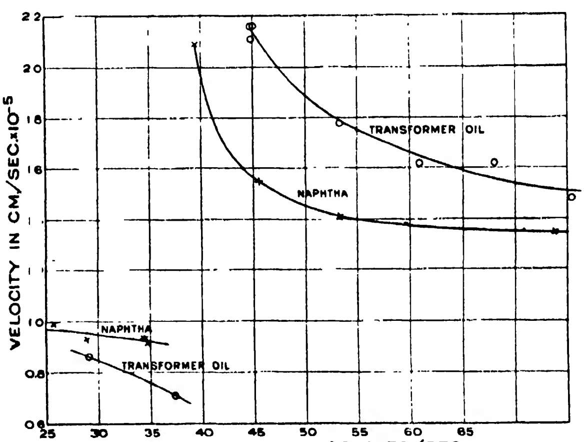

FIG. 8. *Curve of phase velocities for naphtha and transformer oil; transformer oil in glass tube of internal diameter 3.1 cm. and wall thickness, 1 mm. Naphtha in glass tube of internal diameter 3.5 cm., and wall thickness, 2 mm.*

Influence of Diameter of Column

An interesting experiment showed the variation in length of stationary waves along a tube in which the diameter varied.

A conical glass tube of dimensions: internal diameter at large end, 6.60 cm., at small end, 4.80 cm.; length of tube, 39.8 cm.; thickness of glass wall, 2.0 mm., was set up vertically, and a rod oscillator was fitted, as formerly described, into its smaller end. This tube was filled with transformer oil and stationary waves were produced by reflection from a horizontal plate in the oil. When the oscillator was operated at fairly high frequencies no difference could be detected in the lengths of the standing waves, but at a certain lower frequency notable differences in lengths of stationary waves occurred. In Table II the distance between successive nodes with corresponding computed phase velocity is given as well as the mean internal diameter of the tube corresponding to this particular half-wave-length. The results were anticipated from the previous work.

TABLE II

EXPERIMENTAL RESULTS AT A FREQUENCY OF VIBRATION OF 26,170 CYCLES PER SEC.

Distance between nodes half-wave-length in cm.	Corresponding velocity in cm./sec.	Mean diameter over the half-wave in cm.	Distance between nodes half-wave-length in cm.	Corresponding velocity in cm./sec.	Mean diameter over the half-wave in cm.
1.90	0.995×10^5	4.87	3.88	2.03×10^5	5.59
2.00	1.05×10^5	4.36	3.75	1.96×10^5	5.77
2.30	1.20×10^5	5.01	3.58	1.87×10^5	5.93
3.70	1.94×10^5	5.19	3.48	1.82×10^5	6.09
3.88	2.00×10^5	5.37	3.10	1.66×10^5	6.24

In another experiment a glass tube was drawn into the shape shown in Fig. 9.

The internal diameter of the larger sections was 3.0 cm.; that of the smaller 1.7 cm. It was found difficult at most frequencies for stationary waves to form in the smaller section of the tube, but there were three different frequencies at which stationary wave measurements were quite possible. At a frequency of 51,800 cycles per sec. the wave-length in the large sections was 4.40 cm. and in the small section approximately 3 cm.; at 81,700 cycles per sec. the wave-length in the large sections was 1.91 cm. and in the small 2.1 cm.; at 93,600 the wave-length in the large section was 1.67 cm., and in the small 1.86 cm. It is seen clearly from this simple experiment that in a given liquid the phase velocity depends upon (1) the frequency and (2) the diameter of the column, and that the frequency may be so adjusted that the phase velocity is greater in the larger tube than in the smaller, or *vice versa*.

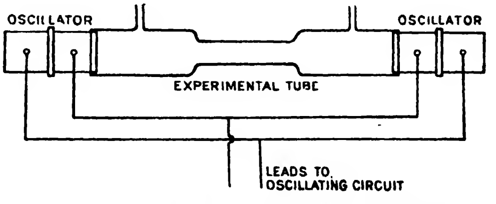

FIG. 9. *Experimental tube of different diameters.*

A series of experiments using transformer oil as the experimental liquid was carried out with glass tubes of the same length and wall thickness, but with different diameters. In Fig. 10 are plotted the frequencies at which the maxi-

mum observable phase velocities occurred in the chosen diameters of tube. On the same sheet has been drawn the curve $n_p = \frac{K}{d}$, where n_p is the absorbing frequency in thousands of cycles per sec., d is the diameter of the tube in cm., and K is a constant (in this case having the value 13.6). It will be observed that a smooth curve drawn through the experimental points would lie approximately on the curve which has been drawn. It is very difficult to obtain n_p experimentally, solely from velocity measurements, since the velocity is asymptotic to the ordinate at the frequency n_p, and the greatest observable velocity may be at a frequency somewhat removed from n_p. But from Fig. 10 there is a striking indication that *the absorbing frequency depends inversely on the diameter of the tube.*

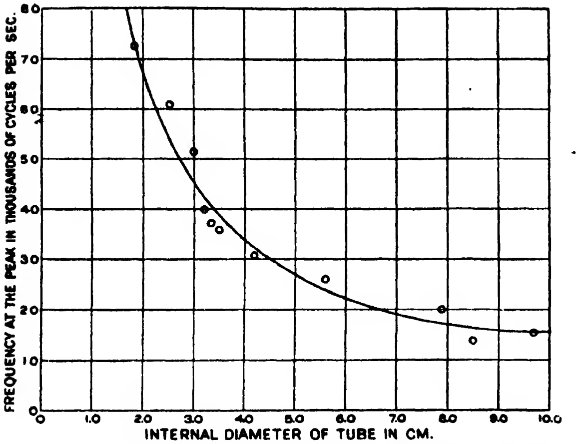

FIG. 10. *Curve showing relation between absorption frequency and internal diameter of tube; transformer oil in glass tubes. The smooth curve represents the relation, peak frequency* $= \frac{K}{\textit{diameter of tube}}$, *where K was taken as 13.6. The observed frequencies were plotted as circles.*

Influence of Length of Tube

To determine whether or not longitudinal resonance in the tube wall, as suggested by Dörsing, had any significant influence on this phenomenon or was a possible cause of the marked energy absorption, special experiments were begun with a piece of glass tubing, about 56 cm. long. This tube was shortened many times and the velocity-frequency curve for the contained liquid was retaken at each length. The shortening was by amounts of about $\frac{1}{8}$ wave-length of longitudinal wave in the wall, at the peak frequency of the velocity curve, for a range over a full wave-length, and after that at irregular intervals, down to a length of 30 cm. It was noted that the velocity-frequency curve remained exactly the same for all the lengths of experimental tube. Herein is a variance from the observations of Dörsing (7), though it must be remembered that he experimented with liquid columns at the much lower frequencies of about 4000 cycles per sec. Dörsing suggested that the vibrating liquid columns generate sympathetic longitudinal vibrations in the tube wall, as undoubtedly they may do, and that to obtain Kundt's dust figures one must make the natural frequencies of liquid and tube as nearly equal as possible. The length of the experimental tube in relation to the length of the wave within the wall or within the contained liquid made no difference here to the formation of the stationary waves, as indicated by the bubbles produced by cavitation in the nodal planes.

A special experiment was later performed with another glass tube, internal diameter 3.1 cm., using oscillators which fitted very snugly into the ends of the experimental tube and which were driven with maximum power at their fundamental resonant frequency; and it was arranged that this frequency was the same as the frequency of the maximum of the velocity-frequency curve. Under such conditions vibrations must have been very readily communicated to the tube walls, but there were no noticeable differences in the experimental results. (The experimental liquid in this particular case was transformer oil.) If there were any shift of the velocity peak for different lengths of tube it was very small indeed, and could not account for the *large difference in peak velocities noticed for tubes of different diameters.*

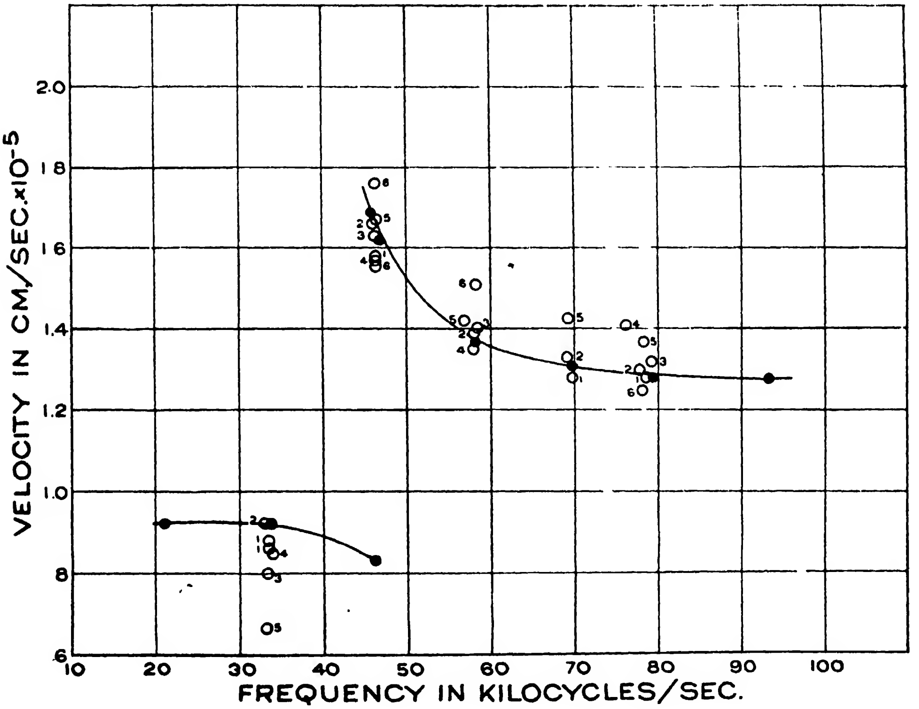

FIG. 11. *Curve showing effect of varying length of tube on the phase velocity. Naphtha in glass tubes, 3.1 cm. internal diameter; wall thickness, 1.4 mm. Points represent length of tube as follows:—*

25 cm. (●), 18.5 cm. (O1), 13.4 cm. (O2), 9.1 cm. (O3), 6.6 cm. (O4), 4.5 cm. (O5), 3.2 cm. (O6).

Very short tubes are commonly employed in sonic interferometer measurements, and it was desired to ascertain definitely whether the velocity-frequency curve would remain unchanged down to extremely short lengths of tube. Hence it was decided to measure velocities in tubes of even shorter length than those formerly used. The liquid employed was lighting naphtha contained in glass tubes of internal diameter 3.2 cm. and greatest length 25 cm. The experimental results are presented in graphical form in Fig. 11.

It will be noticed that down to a tube length of 9.1 cm. (points marked "3" on curve) the observed velocities are the same as those obtained with a tube length of 25 cm., but below 9.1 cm. tube-length, the points depart from the 25-cm. curve in an erratic manner. The rise at 46,000 cycles per sec. however, is quite well defined in all cases, so that the frequency of the absorption band seems to persist unchanged right down to a tube length of 3.2 cm. The deviations from the 25-cm. curve at the higher frequencies are in all probability due to the small number of wave-lengths between the opposing oscillator faces, and the consequent difficulty in accurately measuring wave-lengths.

Effect of Different Thicknesses and Materials of Tube Walls

To determine what effect, if any, the thickness and material of the tube wall had upon the velocity in the liquid, a number of experiments were conducted. Four glass tubes of the same internal diameter, 3.1 cm., and of ordinary thin but different wall thicknesses were obtained and readings of velocity were taken in transformer oil at frequencies near that of the maximum. In all cases the velocity- frequency curve was the same.

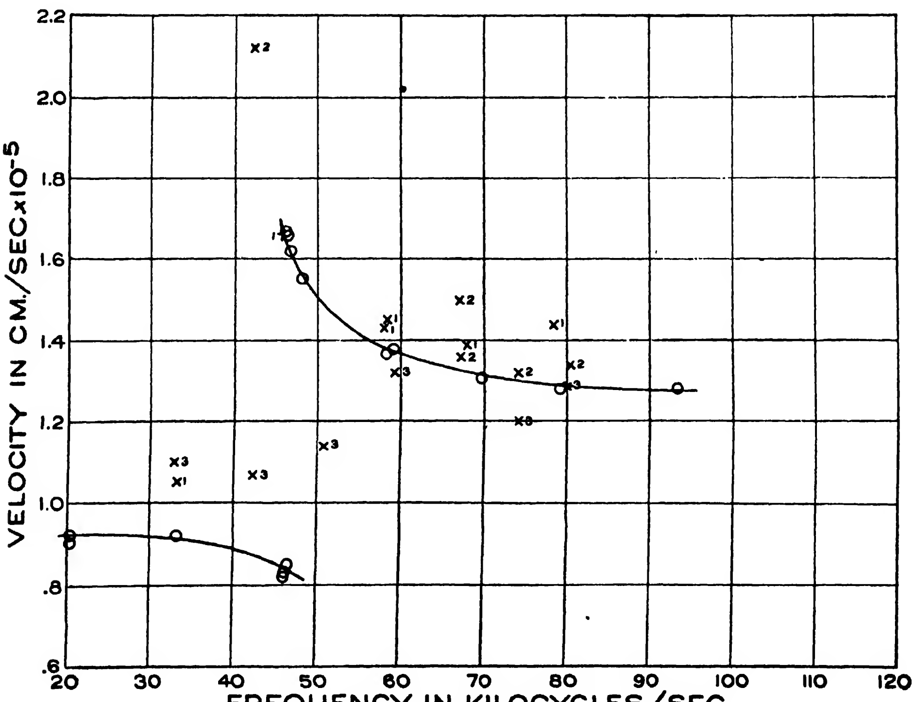

FIG. 12. *Curve showing influence of wall thickness on phase velocity. Naphtha in glass tube; internal diameter of tube, 3.1-3.2 cm. Wall thickness, 1.4 mm.;* $\frac{h}{a} = 0.087$, *points plotted as* (○). *Wall thickness, 2.7-3.0 mm.;* $\frac{h}{a} = 0.18$; *points plotted as* (X1) *and* (X2), *the numerals indicating that the readings were taken on different days. Wall thickness, 0.33 mm.;* $\frac{h}{a} = 0.21$, *points plotted as* (X3). *In this case the nodes were scarcely defined at all, and the readings are very doubtful.*

A thin-walled celluloid tube and a very thin cellophane tube were constructed and filled with lighting naphtha. Readings of velocity were taken and compared with those taken in a glass tube of the same internal diameter but different wall thickness. In these cases also there was no change.

Further readings were taken when using lighting naphtha in tubes of glass, two with quite thin walls and two with comparatively thick walls. Here different results were obtained. The calculated velocities are plotted in Figs. 12 and 13.

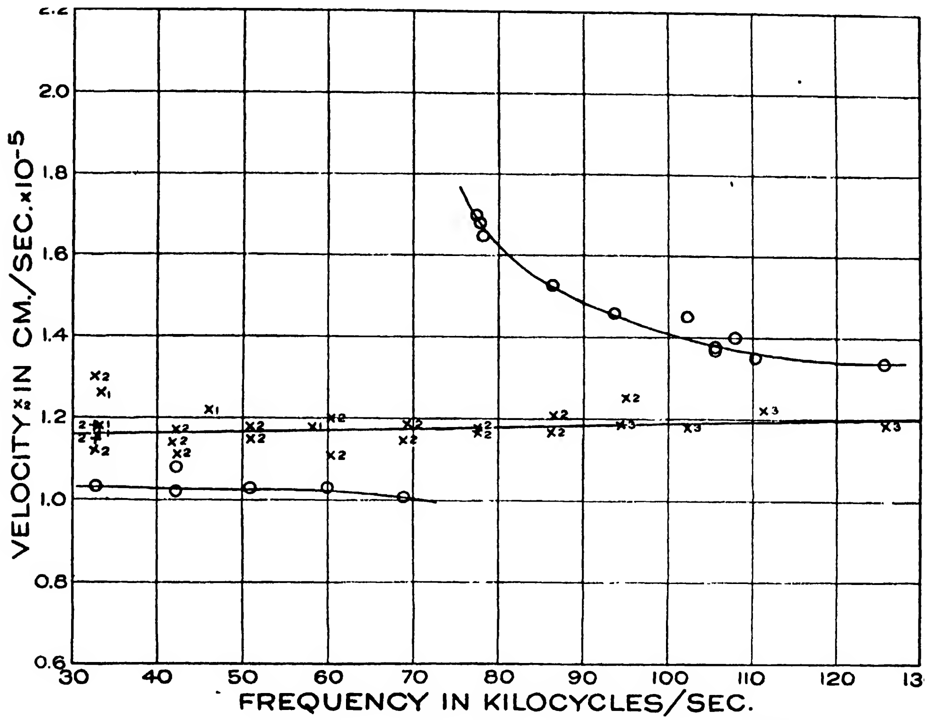

FIG. 13. *Curve showing influence of wall thickness on phase velocity. Naphtha in glass tubes of internal diameter 1.9 cm.; wall thickness, 1.2 mm.;* $\frac{h}{a} = 0.13$*; points plotted as circles. Wall thickness, 3.3 mm.,* $\frac{h}{a} = 0.33$*; points plotted as crosses. The readings were taken on different days as indicated by the numerals alongside the crosses.*

Evidently in wide tubes of relatively thin walls the thickness of the wall and its material has no influence on the absorption frequency and the velocity; but with a given tube material, *when the wall thickness becomes appreciable in comparison with the radius of the liquid column*, the whole phenomenon is greatly affected. The standing wave system is very unstable and velocities are difficult to measure, and seem to be about midway between the low velocity value at lower frequencies and the asymptotic value of velocity at higher frequencies.

These results are very interesting. In Fig. 12, the velocity curve is quite

regular for a ratio of wall thickness (h) to radius (a) of 0.087 $\left(= \frac{0.14}{1.6}\right)$. When this ratio equals 0.18 $\left(= \frac{0.29}{1.6}\right)$ the velocity becomes erratic though the absorption peak is unaltered. For a value of the ratio of 0.21 $\left(= \frac{0.33}{1.6}\right)$, the peak has almost disappeared and the velocity is very difficult to measure.

In Fig. 13 the velocity curve is regular for $\frac{h}{a}$ equal to 0.13 $\left(= \frac{0.12}{0.95}\right)$. When this ratio equals 0.35 $\left(= \frac{0.33}{0.95}\right)$ the velocity shows no trace of a peak and the velocity, although erratic, is beginning to steady down. Further work will be necessary to elucidate this point.

Further Check on Possibility of Lateral Resonance in Tube Wall

As a further experiment to determine whether or not lateral resonance in the tube wall might be affecting the velocity in the contained liquid, it was decided to use tourmaline instead of quartz, as the active piezo-electric material of the rod oscillators. The reason for using tourmaline is that this crystal vibrates in only one main direction of oscillation for a voltage applied in that direction, while quartz may vibrate in three. The tourmaline oscillators, therefore, reduced to a minimum the chance of a lateral vibration being transmitted from the oscillator rods to the tube walls.

The velocities obtained with quartz and tourmaline oscillators were compared, and it was observed that the same velocities in the experimental liquids occurred in both cases, so that the possibility of lateral resonance in the tube wall having anything to do with the absorption phenomenon under observation is very slight.

Absorption at Overtone Frequencies

Attempts were made to find other peaks ("absorption bands") in the velocity-frequency curve. It was thought that if other peaks existed in the curve such might occur at overtones of the frequency of the first, since the effect discussed here was no doubt due to selective resonance of oscillation in some possible degree of freedom. Accordingly in a special experiment, in a glass tube containing naphtha, readings of velocity and frequency were taken up to quite a high frequency. The resulting curve is shown in Fig. 14. It will be seen that the first absorp-

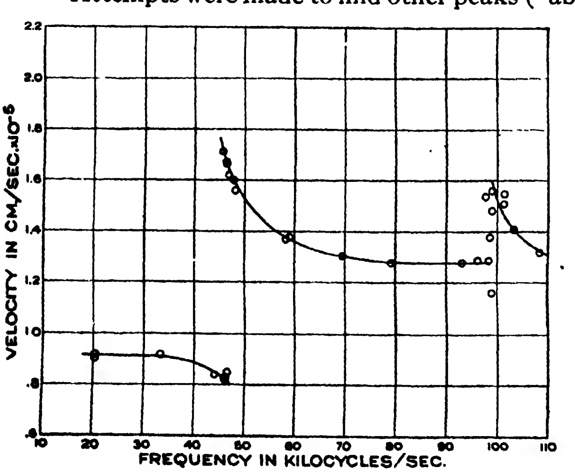

FIG. 14. *Curve showing first and second absorption bands: naphtha in glass tube, 3.1 cm., internal diameter; wall thickness, 1.4 mm.*

tion band existed at a frequency of about 45,000 cycles per sec., and that there is a second band at a frequency of about 100,000 cycles. The depressed velocity observed before the first absorption band seemed to be missing, but the sharp fall in velocity after the absorption frequency is as usual. The experiment shows clearly that more than one absorption frequency, probably existing in a frequency series, may occur in a liquid in the same tube.

Incidental

Velocity in Air

The velocity of sound in air was measured in several tubes by using a hollow steel reflector with attached stethoscope as in the earlier experiments with liquids. No differences in the velocity in the air columns could be found at different frequencies, and the velocities so measured agreed with the unconfined velocity in air at the same temperature. This, however, does not exclude the possibility of selective absorption bands occurring in a gas column when other relations between the nature of the gas, diameter of column, and wave-length may prevail.

Conclusion of Part I

It is evident from these experiments that at certain particular frequencies, for which there exists some relation between the diameter of the tube and the wave-length of the oscillation in the fluid column enclosed by the tube, there is a marked selective absorption of energy which displays all the usual characteristics of an energy absorption band. Phase-velocity is greatly lowered immediately on the lower side and greatly enhanced immediately on the higher side of the critical frequency. Similar would be the conditions of amplitude, particle velocity and harmonic pressure in the waves. No doubt it is at frequencies far removed from the absorption where the wave-length is either very large or very small compared with the diameter of the column that all other experiments on velocity have been carried out; *i.e.*, on the regular and flat portions of the velocity-frequency curves, far removed from the frequency of the discontinuity. It is, no doubt, in these ranges that theories like the Helmholtz-Kirckhoff theory may be applied. At or near the frequency of absorption the velocity changes represented by such theories are relatively insignificant.

The fact that the absorption frequency does not depend on the material or length of the tube, or (for thin walls) on the wall thickness, indicates that it is neither longitudinal nor flexural (lateral) vibrations in the tube walls which cause the phenomenon; and the fact that for any liquid the critical frequency shifts with change of diameter indicates that it is in the column of liquid itself that the energy absorption or transference takes place.

References

1. Boyle, R. W. Nature, 120: 476-477. 1927.
2. Boyle, R. W., Froman, D. K. Nature 126: 602. 1930.
3. Boyle, R. W., Lehmann, J. F. and Reid, C. D. Trans. Roy. Soc. Can. III, 19: 167-196. 1925.

4. Boyle, R. W., Taylor, G. B. and Froman, D. K. Trans. Roy. Soc. Can. III, 23: 187-201. 1929.
5. Busse, W. Ann. Physik, 75: 657-664. 1924.
6. Cornish, R. E. and Eastman, E. D. Phys. Rev. 33: 90-96, 258-259. 1929.
7. Dörsing, K. Ann. Physik, 25: 227-251. 1908.
8. Green, H. G. Phil. Mag. 45: 907-918. 1923.
9. Gronwall, T. H. Phys. Rev. 30: 71-83. 1927.
10. Hubbard, J. C. and Loomis, A. L. Phil. Mag. 5: 1177-1190. 1928.
11. Lamb, H. Mem. Manchester Phil. and Lit. Soc. 42, No. 9, 1898.
12. Lamb, H. The dynamical theory of sound. 2d ed. E. Arnold and Co. 1925.
13. Pooler, L. G. Phys. Rev. 31: 157-158. 1928.

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THE MILLING AND BAKING QUALITY OF FROSTED WHEAT OF THE 1928 CROP[1]

BY W. F. GEDDES[2], J. G. MALLOCH[3] AND R. K. LARMOUR[4]

Abstract

Owing to limited rainfall following germination, combined with late heavy frosts, a large portion of the 1928 wheat crop of Western Canada contained many types of frost damage together with immature and green kernels. A survey of the crop was made in the three laboratories collaborating with the Associate Committee on Grain Research with the dual object of studying the Canadian grading system as applied to frosted wheat and of securing information on the relative effects of green, immature, and frosted kernels on milling and baking quality. The study is based on 228 samples grading from No. 1 Northern to No. 6.

Physical examinations showed that the percentage of sound kernels progressively decreased with a decrease in grade, with a greater relative increase in the percentage of "heavy damage" in the lower grades. Test weight per bushel also decreased. Partial correlations showed that individually the various forms of damage had only a slight effect on reducing weight per bushel, heavily frosted and immature kernels having a greater influence than bran frosted kernels.

On a regrading of the samples after storage over winter, 83.3% of the samples were unchanged in grade while 13.2% received a higher grade.

The mean total flour yield decreased with grade, the variability in yield being much higher within the commercial grades. Owing to the tough and fibrous nature of the middlings there was approximately a 20% increase in the time required to mill a sample of No. 5 or No. 6 wheat as compared with the statutory grades. Bran frost, heavy frost, and immature kernels are negatively correlated with flour yield and are of approximately equal importance in their effects. Weight per measured bushel and the percentage of total sound kernels are the best single indices of flour yield.

Baking quality was determined in the three laboratories using either a 55% patent or a straight grade flour and baking according to the simple, bromate, malt and blend formulas. While the simple formula gave incongruous results all the others revealed that the average baking quality as measured by loaf volume, crumb color and texture decreased with grade except in the instance of grade No. 4 which was superior to No. 3 Northern. Absorption markedly increased in the lower grades. The straight grade and patent flours gave the same relative results when baked by either the simple or the bromate formula.

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The average responses to the differential baking tests also decreased with wheat grade, the magnitude of the individual responses being correlated with protein content. Partial correlations calculated for the response to bromate showed that both protein content and percentage of sound kernels are positively correlated with this variable.

The variability in baking quality within each grade increased with decreasing grade, owing in part to variations in the percentage of sound kernels, but chiefly to variations in protein content. Protein content of wheat is the best single measure of baking quality within each grade, but is not a reliable measure when comparisons are made between grades, owing to differences in protein quality. Partial correlations showed, as in the instance of milling quality, that the various classes of visible damage need not be considered individually with regard to their effects on baking quality.

The results of this study indicate that weight per measured bushel and either the percentage of total sound or hard red vitreous kernels could be used to advantage as grading factors in the commercial grades. It is concluded that the grading system in use in 1928 was applied in an efficient manner and gave a satisfactory indication of the relative quality of the various grades with the exception of the anomalous relationship between No. 3 Northern and No. 4.

The variability in baking quality within grades is excessively high, particularly in the lower grades. Although variations in protein content are chiefly responsible for the lack of uniformity within grades, some improvement may be effected by restricting the variability in the percentage of total sound or vitreous kernels allowable in the various grades. The revisions in the Canada Grain Act instituted in 1930 during the progress of the investigation, are in line with the results of this study and may be expected to bring about a greater uniformity in grade characteristics.

Introduction

In the extensive wheat growing areas of Western Canada the crop is grown under such a wide range of soil and climatic conditions that frequently a portion of the wheat marketed contains green, immature, and frosted kernels. Normally such wheat does not constitute a very large proportion of the total crop, but in the year 1928 a combination of conditions prevailed over the prairie provinces which resulted in a wheat crop of low average quality. Owing to a period of limited rainfall following seeding, germination was not uniform; severe and general frosts occurred in the third week of August. As a result of these two conditions, there were throughout a large proportion of the crop many types of frost damage, combined with green and immature kernels. While the largest wheat crop in the history of the West was produced in that year, the damage was so widespread that 59% of the cars inspected by the Western Division was graded into the commercial grades. As there was no rust epidemic that season, the degrading of the wheat was due principally to frost injury, and resulted in extensive economic losses to the wheat producers of Western Canada.

The widespread occurrence of frosted wheat containing varying percentages of frosted, immature and green kernels provided an excellent opportunity for a study of the influence of these types of damage on milling and baking quality, and in view of its general importance, an extensive investigation of different phases of the problem was undertaken by the Associate Committee on Grain Research as one of its collaborative projects.

In addition to annual surveys of the milling and baking quality of commercial wheat containing frost damaged kernels, the plan of investigation included a study of the quality of wheat artificially frozen at different stages of maturity

in comparison with normal samples grown under the same soil and climatic conditions. Another phase of the problem involved a study of the physico-chemical and chemical changes taking place during maturation of the wheat kernel and the influence of freezing on the course of these changes.

The present report covers the results obtained in a survey of the 1928 crop which was undertaken with the dual object of studying the grading system as applied to frosted wheat, and of securing information on the relative effects of the presence of green, immature and frosted kernels on milling and baking quality.

Experimental

The material used in this investigation consisted of 228 samples of hard red spring wheat grown in various districts throughout the three prairie provinces in the crop year of 1928. Of this number, 14 were composites made up from samples taken for grading and supplied by the Western Grain Inspection division, while the remainder were collected for the Committee by the Canadian Co-operative Wheat Producers Limited through their elevator agents. Fifty-two of the samples originated in Alberta, 138 in Saskatchewan and 24 in Manitoba.

The samples were cleaned, divided into subsamples, using a Boerner sampler, and forwarded to the cereal research laboratories of the Department of Field Crops, University of Alberta; the Department of Chemistry, University of Saskatchewan; and the Department of Agricultural Chemistry, University of Manitoba, for determinations of milling and baking quality. Subsamples were also supplied to the Western Grain Inspection Branch at Winnipeg for grading, and to the Grain Research Laboratory of the Board of Grain Commissioners, Winnipeg, where the physical classification of the kernels was carried out.

I. Physical Characteristics of the Grades

Since the problem was to relate the grading of wheat to its actual milling and baking quality, it seemed logical to examine first the physical characteristics of the wheat in the various grades. Fifty grams of wheat was accordingly weighed from each sample and hand picked into the following classes:

(a) Vitreous—well-matured kernels free from frost damage and starchy spots.

(b) Piebald—well-matured kernels free from frost damage, but having starchy spots.

(c) Starchy—well-matured kernels free from frost damage, but completely starchy.

(d) Bran Frost—kernels showing wrinkling of the bran which did not extend into the crease.

(e) Heavy Frost—all kernels showing frost damage not included in (d).

(f) Immature—fully formed kernels having a dark color; usually referred to as "bronzy" or "pink" kernels.

(g) Green—kernels having a decided green color; usually shrivelled.

After classification, the separates were weighed and the percentage of each calculated. In the instance of some samples there were small quantities of broken kernels, foreign matter, etc., which it was impossible to classify into any of the above groups and these were placed in a separate class.

The physical examinations must be considered as approximate. They were however carried out on the entire series of samples by one individual with frequent rechecking, so that the results may be regarded as fairly consistent.

The weight per measured bushel was determined in one of the collaborating laboratories. For this purpose a container of slightly more than the capacity of the test bucket was filled to overflowing, the excess stroked off with a round strike and the wheat poured into a Cox funnel. This was placed on top of the test bucket and the grain was allowed to run in. After stroking off as before, the weight was taken using a beam of the standard pattern. Since the samples had been stored in the laboratory for some time before these measurements were made, and had dried to a moisture content of approximately 8%, the values obtained for weight per bushel were considerably higher than they would have been at a normal moisture content of 13 to 14%. The samples, however, were quite uniform in moisture content and the weights are thus comparable.

The means for the different classes of kernels and weight per measured bushel are arranged according to grade in Table I. Since only 8 samples graded No. 1 Northern, the results for grades No. 1 Northern and No. 2 Northern were combined. In this table the means for the nondescript class, which was made up of broken kernels etc., have been omitted, thus accounting for the fact that a summation of the percentages of the different classes of kernels does not total 100. For the purpose of statistical study the percentages of vitreous, piebald, and starchy kernels have been grouped together as "Total Sound" and the percentages of heavily frosted, green and immature kernels as "Heavy Damage", and these are also recorded in the table.

Before discussing the relation between physical characteristics and wheat grade it is necessary to consider briefly the Canadian grading system. The regulations governing the statutory grades of red spring wheat are specified in the Canada Grain Act. In 1930, during the progress of this investigation, the definitions were materially amended. The most important changes involved the definition of No. 4 as a statutory grade and an increase in the percentage of hard vitreous kernels required in the different grades. Table II gives the definitions governing the statutory grades in 1925 and also the revisions of 1930. The samples in this study were graded according to the definitions of 1925 and as No. 4 was then a commercial grade it will be referred to as such throughout this report.

The characteristics of the commercial grades No. 4, No. 5, No. 6. and Feed are, however, not laid down in the Canada Grain Act, since the predominating forms of damage vary from year to year. The standards for these grades are established each year by the Grain Standards Board after the crop movement has begun and the prevailing types of damage ascertained. Wheat not eligible for the statutory grades is thus graded into one or other of the commercial grades, by comparison with standard samples depending principally on the type and extent of the prevailing forms of damage.

TABLE I

MEANS, STANDARD DEVIATIONS AND Z VALUES FOR WEIGHT PER MEASURED BUSHEL, AND CLASSES OF KERNELS

Grade	No. of samples	Weight per bush.		Vit-reous	Pie-bald	Star-chy	Total sound (1)		Bran frost (2)		Heavy frost (3)		Immature (4)		Green (5)		H'vy damage (7)	
		Mean lb.	S.D. lb.	Mean %	Mean %	Mean %	Mean %	S.D. %	Mean %	S.D. %	Mean %	S.D. %	Mean %	S.D. %	Mean %	S.D. %	Mean %	S.D. %
1° and 2°	52	64.7	1.3	79.2	5.8	0.4	85.5	6.0	1.9	2.3	0.0	0.0	9.1	4.7	0.3	0.5	9.4	4.7
3°	38	64.3	1.0	64.9	7.7	1.6	74.2	9.9	5.3	4.7	0.4	1.7	15.5	9.5	0.9	0.7	16.8	9.4
4	51	63.7	1.2	50.1	6.4	2.0	58.6	15.2	15.9	11.2	4.2	6.6	15.3	8.3	1 5	1.5	21.1	9.5
5	48	62.5	1.4	24.0	6.2	0.5	30.8	16.5	28.8	17.0	13.2	14 7	19.9	12.8	2 9	3.0	36.0	15.3
6	39	61.3	1.6	16.2	1.4	1.0	18.6	11.1	28.5	15.7	24.9	16.3	18.0	13.5	5.7	5.4	48.5	14.7
Z		1.9309					2.7102		2.5144		1.9060		1 0376		1.6020			
5% Pt.		0.4403					0.4403		0.4403		0.4403		0.4403		0.4403			

NOTE:—*1° = No. 1 Northern; 2° = No. 2 Northern; 3° = No. 3 Northern; 4 = No. 4 Wheat; 5 = No. 5 Wheat; 6 = No. 6 Wheat.*

TABLE II

STATUTORY GRADES OF WESTERN GRAIN—RED SPRING WHEAT

Definitions according to Canada Grain Act 1925*

Number and name of grade	Minimum weight per bushel in lb.	Variety of grain	Percentage of hard vitreous kernels	Standard of quality	Foreign material other than dockage
No. 1 Manitoba Hard	62	Marquis or equal to Marquis	75	Sound	Well cleaned
No. 1 Manitoba Northern	60	Marquis or equal to Marquis	60	Well matured, practically free from damaged kernels	Well cleaned and practically free from foreign grains
No. 2 Manitoba Northern	58	Marquis or equal to Marquis	45	Reasonably sound	Reasonably clean
Or	60	Soft varieties of Red Spring wheat	60	Sound	Reasonably clean, may contain Amber or Red Durum singly or in combination up to 1%
No. 3 Manitoba Northern	57	Red spring wheat varieties fair milling quality		Reasonably sound	Reasonably clean, may contain Amber or Red Durum wheat, singly or in combination up to 3%

Definitions according to Canada Grain Act 1930, Schedule 1.

Number and name of grade	Minimum weight per bushel in lb.	Variety of grain	Percentage by weight of hard vitreous kernels	Standard of quality	Foreign material other than dockage		Wheat of other classes	
					Matter other than cereal grains	Total including cereal grains %	Durums %	Total including Durum %
No. 1 Manitoba Hard	62	Marquis or equal to Marquis	80	Sound and well matured	Free	Free		
No. 1 Manitoba Northern	60	Marquis or equal to Marquis	65	Well matured, practically free from damaged kernels	Free	Practically free	Practically free	1
No. 2 Manitoba Northern	58	Red spring wheat of good milling quality	50	Reasonably well matured, reasonably free from damaged kernels	Free	About 1%	1	3
No. 3 Manitoba Northern	57	Red spring wheat of fair milling quality	25	Reasonably well matured, reasonably free from damaged kernels	Reasonably free	About 2%	3	10
No. 4 Manitoba Northern	57	Red spring wheat		Reasonably well matured, but excluded from preceding grades on account of frosted or otherwise damaged kernels	Reasonably free	About 2½%	4	10
Or	55	Red spring wheat		Rusted or shrunken but otherwise reasonably sound	Reasonably free	About 2½%	4	10

**Arranged in table form by T. J. Harrison, Assistant Grain Commissioner for Manitoba.*

The setting up of standards for the commercial grades of the 1928 crop presented a difficult problem owing to the existence of two types of damaged kernels. As a consequence of uneven germination there was an unusually large percentage of immature and green kernels in the threshed grain, while the general occurrence of late heavy frosts, combined with uneven growth, resulted in many types of frost damage in grain harvested from the one field. In grading such wheat it was therefore necessary to balance one type of damage against another.

The mean values for weight per bushel and classes of kernels recorded in Table I and graphically represented in Fig. 1 show a definite gradation from the higher to the lower grades and it is important, therefore, to have some measure of the significance of this gradation or trend. Since the grade numbers obviously do not represent numerical differences between the grades it is illogical to calculate correlation coefficients for the relations between the means for the physical classes and the grade numbers. The best measure of the significance of the trend is given therefore by the significance of the variance contributed by the differences between the means for the grades as compared to the variance between the samples within the grades. This procedure is identical with that of testing the significance of the correlation ratio. The analysis for each physical class is carried out as in Table III for weight per bushel.

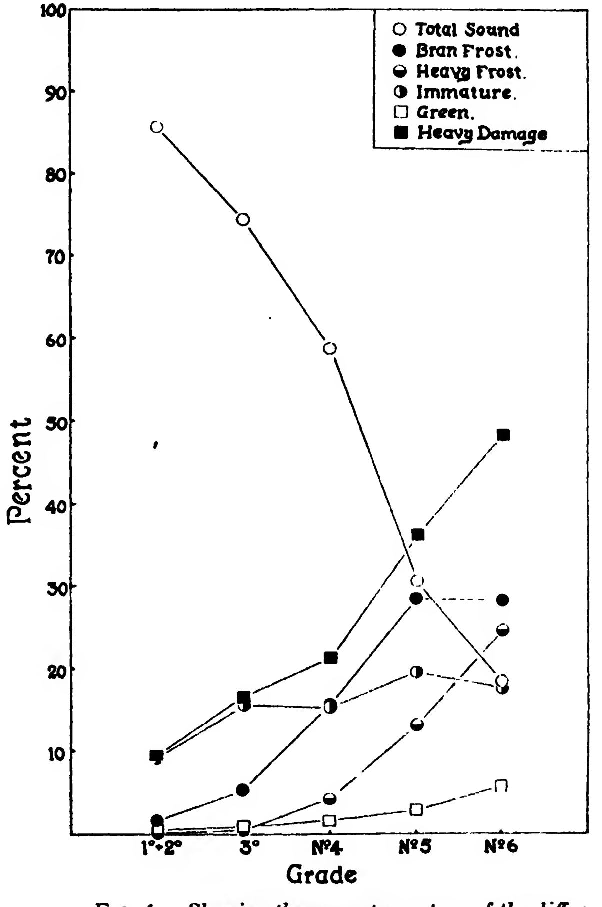

Fig. 1. *Showing the mean percentage of the different classes of kernels according to wheat grade.*

For 4 and 223 degrees of freedom the 5% point for Z is 0.4403 so that the variance between grades is very significantly higher than that within grades. The Z values representing differences between the grades for the means of the physical classes are given with their respective 5% points at the foot of Table I. In every instance the Z values exceed the 5% point, and hence the differences

TABLE III

COMPARISON OF VARIANCE BETWEEN AND WITHIN GRADES FOR WEIGHT PER MEASURED BUSHEL

Variance due to	Sum of squares	Degrees of freedom	Variance	1/2 log e	Z
Between grades	346.06	4	86.52	2.2303	1.9309
Within grades	405.20	223	1.82	0.2994	
Total	751.26				

between the means for weight per measured bushel and the various classes of kernels recorded for the different grades are significant. In other words the grades are clearly differentiated with respect to their physical characteristics.

The total percentage of damaged kernels increases markedly as the grade is lowered. Considering the individual forms of damage, the percentage of bran frost increases from No. 1 Northern to No. 5, and then decreases. This decrease, however, is compensated by the greater relative increase in the percentage of heavy frost in going from Grade No. 5 to Grade No. 6. The percentage of green kernels increases regularly with a decrease in grade.

While the significant trends in the mean values for the classes of kernels in the different grades imply that, in general, the present system of grading according to physical classification is being applied in a satisfactory manner, the grading system must be examined from the point of view of uniformity within each grade.

The range of variability within each grade has been measured for each of the physical characters in terms of the standard deviation. While there is no definite trend in the variability of weight per bushel for the different grades, there is a fairly consistent increase in the variability of the classes of kernels as the grade decreases from No. 1 Northern and 2 Northern to No. 5. Similar trends have been observed in flour yield and baking quality. In these instances their significance has been tested and this question will be discussed more fully later.

Simple correlations have been calculated between the various classes of kernels, and are recorded in Table IV for each grade, and for the entire series. The 5% points are stated at the foot of each section giving the correlation coefficients for a particular grade. These 5% points were determined by the use of R. A. Fisher's tables of t (3). Since all the coefficients involve more than 30 degrees of freedom the t value for the 5% point is approximately 2.0. Then from the formula for t,

$$t = \frac{r\sqrt{n}}{\sqrt{1-r^2}},$$

where n is the number of degrees of freedom, we obtain,

$$r = \frac{2}{\sqrt{n+4}}.$$

To facilitate the interpretation of the table the correlations which are statistically significant have been printed in italics.

In grades No. 1 Northern and No. 2 Northern the percentages of immature and bran frosted kernels, which are generally regarded as the less severe forms of damage, are chiefly responsible for the variation in the percentage of sound kernels. As the grade decreases the magnitude of the negative correlation between total sound and immature progressively decreases, and in grades No. 5 and No. 6 the percentages of heavily frosted kernels become a significant factor in causing a decrease in the percentage of sound kernels. This is indicative of a progressive change in the character of the samples going into each grade.

TABLE IV

SIMPLE CORRELATIONS BETWEEN CLASSES OF KERNELS

Grade	Class of kernel	Bran frost (2)	Heavy frost (3)	Immature (4)	Green (5)
1° and 2°	Total sound (1)	− *.463*	+ .174	− *.848*	− .094
	Bran frost (2)		− .092	+ .032	− .031
	Heavy frost (3)			− .092	+ .021
	Immature (4)				+ .048
		5% Pt. r = .271			
3°	Total sound (1)	− .207	− .017	− *.839*	+ .165
	Bran frost (2)		− .092	− .210	− *.327*
	Heavy frost (3)			− .160	+ .014
	Immature (4)				− .078
		5% Pt. r = .316			
No. 4	Total sound (1)	− *.756*	− .257	− *.569*	− .057
	Bran frost (2)		− 049	+ .151	− .148
	Heavy frost (3)			− 229	− .241
	Immature (4)				+ .212
		5% Pt. r = .275			
No. 5	Total sound (1)	− *.568*	− *.398*	− 109	+ .156
	Bran frost (2)		− 135	− *.344*	− *300*
	Heavy frost (3)			− *.397*	− *.307*
	Immature (4)				+ *.313*
		5% Pt. r = .283			
No. 6	Total sound (1)	− *.386*	− *.410*	+ .046	+ .104
	Bran frost (2)		− .307	− *.441*	+ .105
	Heavy frost (3)			− *.382*	− *.431*
	Immature (4)				− .100
		5% Pt. r = .312			
Entire series	Total sound (1)	− *.780*	− *.671*	− *.402*	− .439
	Bran frost (2)		+ *.311*	+ .043	+ *.286*
	Heavy frost (3)			− .065	+ *.147*
	Immature (4)				+ *.188*
		5% Pt. r = .132			

It will also be observed that for grades No. 5 and No. 6 the percentage of bran frost is negatively correlated with immature kernels. For these grades heavy frost also is negatively correlated with immature and green kernels, indicating that one form of damage is being balanced against another in determining the grade.

TABLE V

SIMPLE, PARTIAL, AND MULTIPLE CORRELATION COEFFICIENTS INVOLVING WEIGHT PER MEASURED BUSHEL AND CLASSES OF KERNELS

	Simple correlation coefficients					Third order partial correlation coefficients	
	Total sound (1)	Bran frost (2)	Heavy frost (3)	Immature (4)	Green (5)		
Weight per bushel (w)	+ .595	− .319	− .446	− .351	− .309	$r_{w.2.345}$	− .158
Total sound (1)		− .780	− .671	− .402	− .439	$r_{w.3.245}$	− .440
Bran frost (2)			+ .311	+ .043	+ .286	$r_{w.4.235}$	− .366
Heavy frost (3)				− .065	+ .147	$r_{w.5.234}$	− .159
Immature (4)					+ .188		
Green (5)							
	At 5% pt. $r = .132$					At 5% pt. $r = .133$	

Multiple $R_{w}.2345 = .625$ 5% pt. = .320

Relation Between Weight per Measured Bushel and Classes of Kernels

From the standpoint of wheat grading it is of interest to examine the relations between the physical characteristics of wheat that affect grading. As shown in Table V, there is a negative correlation between weight per bushel and all of the forms of damage and since "total sound" represents all of the remaining kernels after the damaged ones have been removed, there is a positive correlation between weight per bushel and "total sound". The partial correlations have been determined as shown in the table for weight per bushel with each of the forms of damage when all of the other classes of damage are held constant, and they indicate that heavily frosted and immature kernels have a greater effect on weight per measured bushel than bran frosted or green kernels. It is of interest that, as compared with the simple correlations, the partials involving weight per bushel with bran frost and with green kernels are lower and only barely significant. Green and bran frosted kernels are usually plump and hence should not affect the weight per bushel seriously. The simple correlations are due therefore very largely to the association of these two classes with heavy frost and immaturity.

It is obvious from the nature of the data that the partial correlation $r_{w1.2345}$ would be zero as the sum of 1, 2, 3, 4 and 5 is 100 for all samples except for slight variations due to the very small nondescript class. The multiple $R_{w.2345}$ is analogous to the simple correlation r_{w1} and while of greater magnitude is probably of the same order of significance.

The magnitude of the simple correlation between weight per bushel and percentage of sound kernels, together with the observation that heavily frosted and immature kernels are chiefly responsible for the decrease in weight per bushel, suggests the utility of weight per measured bushel as a grading factor in the commercial grades.

Effect of Storage on Grading

It is believed by some wheat producers that the grain improves in appearance and hence in grade when held in storage over winter. In order to determine whether there was any basis in fact for this belief the entire series of samples which was graded originally in December 1928 were regraded the following July. The changes in grade shown in Table VI in no case exceeded one grade.

TABLE VI

CHANGES IN GRADE DURING STORAGE

Grade in Fall	Number of samples	Samples with changed grade in Spring			
		Higher		Lower	
		Number	%	Number	%
1°	8	0	0.0	2	25.0
2°	44	2	4.5	2	4.5
3°	38	8	21.0	1	2.6
No. 4	51	3	5.7	2	3.8
No. 5	48	7	14.6	1	2.1
No. 6	39	10	25.6	0	0.0
Total	228	30	13.2	8	3.5

The results show that 16.7% of the samples changed in grade, 13.2% receiving a higher grade. The percentage values for the individual grades show that a relatively greater number of samples graded higher in the cases of No. 5 and No. 6. This indicates that there may actually have been a change in physical appearance, particularly in the samples containing a large proportion of damaged kernels. If due allowance is made for borderline samples, the fact that 83.3% of the samples remained unchanged in grade indicates that the consistency of grading is fairly satisfactory for work of this nature where the personal factor plays such an important role.

II. Milling Quality

The commercial value of hard red spring wheat depends upon two factors, the quantity and quality of flour which the wheat is capable of yielding. The first factor, depending upon flour yield, is usually referred to as milling quality, and the latter, which involves flour strength, as baking quality. The classification of wheat into grades, by reference to physical characteristics which may be rapidly determined or estimated, such as weight per bushel and external appearance, is made with the object of classification into groups differing in

wheat quality. The samples in this study were therefore submitted to experimental milling and baking procedures in order to determine the value of such physical characters as are now employed in grading as an index of actual value, and further to ascertain the relative effect of the various forms of damage which occur in increasingly large quantities as the grade decreases.

In experimental milling one of two procedures is usually followed. Either a patent flour, representing some definite percentage of the total flour, or a straight grade which includes all the flour, is milled. When a patent flour is milled the percentage of the total flour it is desired to include is made up from the best portions, and there is some question among cereal chemists as to which is the most desirable procedure from the standpoint of determining the baking quality of a series of wheats.

In this study both procedures were followed, two of the collaborating laboratories milling a 55% patent, and the other essentially a straight grade flour. These two practices were used in order to ascertain whether there would be any marked difference in the relative baking value of the samples when determined by the use of flour of such widely different extractions. It was thought that the relative baking results on these two flours for a series of samples might reveal whether the damage were external or fairly deep-seated. If external, the short patent would be expected to show progressive superiority over the straight grade flour as the percentage of frost damaged kernels increased.

A uniform system of milling was followed in the three laboratories. The wheat samples after cleaning and scouring were conditioned to 13% moisture from four to seven days prior to milling, a 2000-gm. sample was tempered to 15% moisture and milled on an Allis Chalmers experimental mill following the flow sheet published by Geddes (4). To provide for greater uniformity in milling, standard samples of feed flour and shorts were forwarded to each of the collaborating laboratories. Extraction of flour from the shorts was continued until the residue matched the standard and the total flour thus obtained was weighed.

In making up the patent flour, successive fractions obtained by the progressive reduction of the middlings were taken until 55% of the total flour was included. In milling a straight, reduction of the feed flour was continued until the residue matched the standard. In making up the straight, all flour with the exception of residual feed flour was included. The term straight grade flour is hence not strictly correct as in reality a long patent was milled. However, in order to differentiate clearly between the two extractions, the long patent is referred to as a straight grade flour. The mill rooms were maintained at a relative humidity of approximately 70% by means of Bahnson humidifiers. Two of the laboratories were equipped with three-stand experimental mills, and the other, designated as Laboratory A, used a two-stand mill by the same manufacturer. Each laboratory was supplied with 60 lb. of the same lot of 2° wheat, obtained from the Winnipeg Grain Inspection office, which served as a milling standard.

By expressing the total flour yields obtained in each laboratory as a percentage of the mean value for replicate millings of the standard wheat, it was possible to determine whether the different laboratories obtained the same relative results on a particular sample, thus facilitating compilation of the data. Such a procedure was necessary because of considerable differences in the absolute values obtained, the total flour yields reported by Laboratory A being consistently lower. This may have been due in part to the technique of the operator, but more likely to the fact that this laboratory was equipped with a two-stand mill, and probably did not secure as thorough a clean up of the bran. However, in general, the three laboratories obtained the same relative results with the exception that Laboratory A secured relatively lower flour yields for Grade No. 6. For this reason the total flour yields obtained in that laboratory have been recorded separately, in addition to the average values for the three laboratories. In milling according to the method outlined above, it was observed that the yield of straight grade flour did not approximate a constant percentage of the total but progressively decreased as the milling quality of the samples became lower. That is, in milling the lower grade wheats an increasing amount of feed flour which matched the arbitrary standard was obtained. In view of this observation the yield of straight grade flour has been expressed as a percentage of the total flour obtained for Laboratory A. Protein determinations were made on the wheat by the usual Kjeldahl-Gunning procedure and the results expressed on a 13.5% moisture basis.

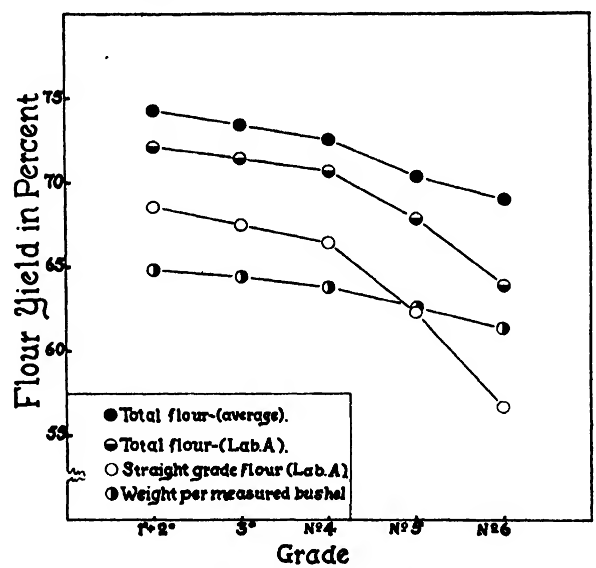

FIG. 2. *Showing the mean yield of total flour, straight grade flour, and mean weight per measured bushel according to wheat grade.*

The mean values for wheat protein and milling results summarized according to wheat grade are given in Table VII, together with the data for weight per measured bushel previously given in Table I. The significances of the trends in the mean values have been determined by analyses of variance and are indicated by the Z values and their respective 5% points at the foot of Table VII. The range of variability within each grade in terms of the standard deviation is also recorded for each set of values.

There is, as might be expected, no significant difference in the mean protein content of the samples in the various grades, but the decrease in the yield of

both total and straight grade flour as the grade is lowered is highly significant. The trends in flour yield and weight per bushel are represented graphically in Fig. 2, and show that Laboratory A, despite a lower flour yield, obtained the same relative results as the three laboratories combined with the exception of the values for grade No. 6.

Before considering the trends in milling quality for the different grades it seemed advisable to determine the extent of the relationship between the three sets of values for flour yield given in Table VII. Simple correlations have accordingly been computed between the percentages of straight flour and the two values for total flour yield. The results by grades and for all grades combined are recorded in Table VIII. It will be noted that there is a very close relationship between the yield of straight grade flour and that of total flour obtained in the same laboratory. The straight grade flour yield is also highly correlated with the average total flour yield of the three laboratories, being however somewhat lower for grade No. 6 as is evident from Fig. 2.

The covariance analysis given in the lower half of Table VIII is useful in showing the relation between the correlated variables, throughout the entire population of samples, for the means of the variables for the corresponding grades, and within the grades. It will be noted that there is a very close relation between the mean, total and straight grade flour yields for the different grades and that there is a very significant relation between these variables

TABLE VII

MEANS, STANDARD DEVIATIONS AND Z VALUES FOR WEIGHT PER MEASURED BUSHEL, PROTEIN CONTENT OF WHEAT, AND FLOUR YIELD

Grade	No. of samples	Weight per measured bushel		Protein in wheat (13.5% M.B.)		Total flour yield. Av. all labs.		Total flour yield Lab. A		Str. grade flour yield Lab. A		Str. grade flour as % total
		Mean lb.	S.D. lb.	Mean %	S.D. %	Mean %	S.D. %	Mean %	S.D. %	Mean %	S.D. %	Mean %
1° & 2°	52	64.7	1.3	12.2	1.1	74.0	1.2	72.0	2.0	68.5	2.6	95.1
3°	38	64.3	1.0	11.8	1.2	73.4	1.7	71.4	1.8	67.4	2.0	94.4
No. 4	51	63.7	1.2	12.1	1.2	72.6	1.4	70.6	2.2	66.3	2.3	93.9
No. 5	48	62.5	1.4	12.0	1.3	70.3	1.9	67.6	2.5	62.1	4.1	91.9
No. 6	39	61.3	1.6	12.3	0.9	69.0	2.6	63.8	3.7	56.5	5.0	88.6
Z		1.9309		0.0585		2.0488		2.1662		2.3470		
5% pt.		0.4403		0.4403		0.4403		0.4403		0.4403		

within the grades. The correlation within grades may be considered an estimate of the correlation to be expected within any one grade on the assumption that the actual variations in this value for the different grades are due merely to random sampling. In view of these high and significant correlations, the different measures of milling quality show good agreement and any one set of results for flour yield may be taken as a measure of the relative milling value of the different grades.

TABLE VIII

SIMPLE CORRELATIONS BETWEEN DIFFERENT MEASURES OF MILLING QUALITY

Correlations between	Simple correlation coefficients—wheat grades					
	1° & 2°	3°	No. 4	No. 5	No. 6	All grades
Total flour yield av. all labs. (t_1) and straight flour yield (s). r_{t_1s}	+ .693	+ .625	+ 515	+ .642	+ .433	+ 793
Total flour yield lab. A (t_2) and straight flour yield (s). r_{t_2s}	+ .934	+ .911	+ .855	+ .811	+ .953	+ .960
5% Pt.	.271	.316	.275	.283	.312	.132

Analysis of Covariance for Total Flour Yield (Av.) and Total Flour Yield (Lab. A), with Yield of Straight Grade Flour (Lab. A)

Correlation coefficients for	Straight grade and total flour av.		Straight grade and total flour Lab. A		5% Pt.	Degrees of freedom
	r_{st_1}	t	r_{st_2}	t		
Total, all grades	+ .793	19.5	+ .960	51.9	1.96	226
Between grades	+ .949	5.2	+ .970	6.2	3.18	3
Within grades	+ .586	10.7	+ .807	20.2	1.96	218

The decrease in total and straight grade flour yield is quite uniform to grade No. 4 and then decreases sharply in the lower commercial grades. The falling-off in the yield of straight flour is most marked, the spread between the top and bottom grades being 12.0% as compared with 8.2% for the yield of total flour obtained by the same laboratory. This implies that in commercial practice a greater proportion of the flour from the lower grade wheats would have to be excluded from the top patents in order to obtain flours approximating in color and ash content those obtained from the higher grades. While the spread in flour yield obtained commercially between the top and bottom grades probably differs somewhat in magnitude from that obtained experimentally, because of the more refined machinery employed, the experimental milling results show clearly that the grading system gives on the average a satisfactory differentiation of wheat on the basis of milling quality.

It should also be mentioned that considerable difficulty was experienced in the reduction of the middlings in the instance of low grade wheats. Owing to the tough and fibrous nature of the middlings, there was approximately a 20% increase in the time required to mill a sample of No. 5 and No. 6 wheat as compared with the statutory grades. This implies that in commercial practice the output of the mill would be lowered and the overhead cost per barrel of flour increased when low grade wheats are milled.

Aside from milling yield, the color and ash content of the flour are important factors in its commercial valuation. At the present time color of flour cannot

readily be evaluated quantitatively, and hence in this study no effort has been made to investigate this factor. It was noted, however, that, in general, the flours from the lower grades of wheat were distinctly inferior in color to those of the top grades. The wheats in grades No. 5 and No. 6 for the most part yielded flours possessing a "dull" or grey color, while the flours from the higher grades were "creamy" or "creamy yellow".

The ash content of several samples of straight flour was determined by the "straight ash" method (A.O.A.C. method) of the American Association of Cereal Chemists (1). The mean results expressed on a 13.5% moisture basis are given in Table IX. The Z value exceeds the 5% point and hence the differences in the mean values are significant. This is due to the higher ash content of flours milled from grade No. 5 and No. 6 wheats as the means for the other grades are identical. The range is only 0.03% and would probably have been greater had the straight grade flour represented a constant percentage of the total for the different grades. The data indicate, however, that it would be necessary to include still less of the total flour in the straight, in the instance of low grade wheats, in order to approximate the ash content of the flours milled from the higher grades. The difference in the commercial value of the various grades from the standpoint of milling quality may hence actually be greater than that indicated by the ranges in flour yield obtained experimentally by the methods employed in this study.

TABLE IX

MEAN ASH CONTENT OF STRAIGHT GRADE FLOURS

Grade	Number of samples analyzed	% Ash (13.5% m.b.)
1° and 2°	21	0.46
3°	13	0.46
No. 4	21	0.46
No. 5	30	0.49
No. 6	20	0.49
Z		0.8248
5% Point		0.4507

While a study of the mean flour yields of the several grades reveals that they are significantly different in respect to milling quality, the variability in flour yield within a grade is also of importance, not only to the miller, but particularly to the wheat producer who is interested in the grading of his own relatively small parcel of wheat.

The standard deviations given in Table VII show that the general tendency is for variability to increase in the lower grades and this increase is especially marked for grade No. 6. A suitable test for the trend in the standard deviations from the higher to the lower grades is somewhat difficult to devise as the grade numbers do not represent numerical values, but it is possible to test any one standard deviation against another or to arrange them in groups and test one group against another. A logical method is to place all the contract grades in one group and all of the commercial grades in another. This has been done for the variability in total flour yield obtained by Laboratory A and also for the three laboratories combined. The results of the test are given in Table X in terms of Z values and their 5% points. The Z values are quite significant, showing that the variability in flour yield is appreciably higher in the commercial than in the contract grades.

Relation between Physical Characteristics of Wheat and Milling Quality

The relation between test weight per bushel and flour yield is shown by the simple correlation coefficients given in Table XI. The correlations between total flour yield, straight flour yield, and weight per measured bushel for the entire series are very significant and their magnitude reveals, in agreement with numerous reports in the literature, that test weight per bushel is a valuable index of flour yield. The correlations for individual grades are much lower and in some instances are not significant. Hence the magnitude of the total correlation is due largely to the differences between the grades. In other words the correlation between the mean flour yields and mean weight per bushel for the five grades must be high. This is obvious from Fig. 2.

TABLE X

COMPARISON OF VARIABILITY IN TOTAL FLOUR YIELD IN THE CONTRACT AND COMMERCIAL GRADES

Grades	Sums of squares total flour yield		Degrees of freedom	Variance	1/2 log e	Z	5% Pt.
All laboratories							
1° & 2°	81.0	192.0	88	2.182	0.3900	0.3047	0.1632
3°	111.0						
No. 4	101.0	541.6	135	4.012	0.6947		
No. 5	177.6						
No. 6	263.0						
Laboratory A							
1° & 2°	203.8	333.7	88	3.791	0.6664	0.4388	0.1632
3°	129.9						
No. 4	248.8	1105.7	135	8.193	1.1052		
No. 5	311.1						
No. 6	545.8						

Since weight per bushel is not an important factor in grading wheat into the commercial grades Nos. 4, 5, and 6, it is of interest to examine the correlations between weight per bushel and flour yields within the grades. For the average total flour yield the correlation falls off slightly from grades 1° and 2° to No. 6, but for yield of straight grade flour there is no evidence of such a trend. Also, in referring to Fig. 2 it will be observed that the drop in weight per bushel from No. 4 to No. 6 is even greater than from grades 1° and 2° to No. 4. Consequently, from the standpoint of flour yield, weight per bushel is just as useful a factor in determining the lower grades as it is in determining the higher grades.

TABLE XI

SIMPLE CORRELATIONS BETWEEN WEIGHT PER MEASURED BUSHEL AND FLOUR YIELD

Correlations between	Simple correlation coefficients. Wheat grades					
	1° & 2°	3°	No. 4	No. 5	No. 6	All grades
Weight per bu. (w) and total flour yield. Av. all labs. (t); r_{wt}	+ .507	+ .279	+ .146	+ .266	+ .338	+ .641
Weight per bu. (w) and straight flour yield. Lab. A (s); r_{ws}	+ .398	+ .302	+ .503	+ .284	+.566	+ .723
5% Pt.	.271	.316	.275	.283	.312	.132

The simple, fourth order partial and multiple correlations for all grades combined, given in Table XII, summarize the relation between flour yield, weight per bushel and classes of kernels. It is obvious from this table that flour yield is closely and positively correlated with weight per bushel and also with percentage of sound kernels, and that these physical characteristics are practically equal in value as a basis for the classification of wheat samples with respect to flour yield.

TABLE XII

SIMPLE, FOURTH ORDER PARTIAL AND MULTIPLE CORRELATIONS BETWEEN FLOUR YIELD AND PHYSICAL CHARACTERISTICS OF WHEAT

	Simple correlations		Fourth order partial correlations			
	Total flour yield av. all labs. (t)	Straight grade flour yield (s)	Total flour yield average all labs. (t)		Straight grade flour (s)	
Total sound (1)	+ .716	+ .717				
Bran frost (2)	− .459	− .515	$r_{t2.345w}$	− .318	$r_{s2.345w}$	− .388
Heavy frost (3)	− .561	− .546	$r_{t3.245w}$	− .467	$r_{s3\ 245w}$	− .388
Immature (4)	− .395	− .349	$r_{t4.235w}$	− .408	$r_{s4.235w}$	− .293
Green (5)	− .312	− .384	$r_{t5.234w}$	− .074	$r_{s5.234w}$	− .172
Weight per bushel (w)	+ .641	+ .722	$r_{tw.2345}$	+ .337	$r_{sw\ 2345}$	+ .507
	At 5% Pt. = .132		At 5% Pt. r = .133			

MULTIPLE CORRELATIONS			R	5% point
	$R_{t(2345w)}$	=	.794	.322
	$R_{s(2345w)}$	=	.832	.322

The partial correlations of the fourth order measure the relationship between milling quality, test weight per bushel, and forms of damage, when each of the factors varies alone. It will be noted that bran frost, heavy frost, and immature kernels are negatively correlated with flour yield and are of approximately equal importance in their effects. As would perhaps be expected, the green kernels have very little effect on flour yield when the other variables are held constant. The multiple correlations are quite high and show that the various forms of damage combined with test weight per bushel give a very good indication of flour yield.

Since the forms of damage (2, 3, 4, and 5) are the complement of "total sound", and the effects of each class of damage when varied alone are practically equal and of a low order, it is evident that the percentage of sound kernels without regard to the particular forms of damage present, together with weight per measured bushel, gives the best indication of the probable milling value. While the data suggest that green kernels have somewhat less influence on flour yield than the other classes of damaged kernels, they normally constitute, as may be seen from Table I, such a small proportion of the total damage that no appreciable error would be expected by including them with the other forms of damage without special consideration.

III BAKING QUALITY

"Baking quality" is used here synonymously with flour strength as a term to designate in a general way those characteristics of a flour which make it desirable for bread making purposes. The experimental determination of the quality of Canadian wheat in so far as flour strength is concerned presents a difficult problem. A particular parcel of wheat under investigation is almost invariably mixed for domestic consumption with other lots of hard red spring wheat, or blended with a wide variety of softer wheats if sold on the foreign market. Furthermore, the resulting flours are baked under widely varying conditions with regard to baking formulas, methods of mixing and fermentation time. Finally the character of the loaf desired differs according to country and locality.

The problem of evaluating the baking quality of Canadian wheat is simplified, however, by the fact that sound Marquis wheat from the southern portions of the prairie provinces is universally recognized as the standard of quality, and such wheat thus provides a standard on which to base comparisons. Until recently the baking formula in most common use in cereal laboratories included only flour, water, yeast, sugar and salt as ingredients, but Larmour and MacLeod (8, 9) have adduced abundant evidence that such a formula often gives incongruous results when applied to flours experimentally milled from Western Canadian wheat. The addition of potassium bromate to the formula, or a blend of the flour under study with a weak flour, however, gave results much more consistent with the commercial evaluation of Canadian wheats.

In view of these considerations it has been deemed essential, in the collaborative work of the Associate Committee on Grain Research, to apply different baking formulas which are designed to reveal the baking characteristics of the flours under several representative conditions. The formulas used in the present study were as follows:

1. Unblended Flour

- (a) "Simple" formula—including only flour, water, yeast, sugar and salt.
- (b) "Bromate" formula—Simple formula with the addition of 0.001 gm. potassium bromate, an improver which affects gas retention.
- (c) "Malt" formula—Simple formula with the addition of 1.0 gm. Fleischmann's diastatic malt, which affects gas production.

2. Blended Flour

"Blend" formula—the flour under investigation was blended with 10% corn starch and baked by the bromate formula.

In the blend formula corn starch was used since it has been found to give results essentially similar to the addition of 40% of a weak flour and has the advantage of being more uniform in composition. The blend was baked by the bromate formula to compensate for the limited aging which experimentally milled flours receive before being submitted to the baking test.

Aside from variations in the baking formulas the baking procedure is standardized and is similar, with certain minor modifications, to the tentative "Basic Standard Procedure" of the American Association of Cereal Chemists as outlined by Blish (2). The basic procedure is followed with respect to the proportions of yeast, salt, and sugar, with the difference that 100 gm. of flour on a 13.5% moisture basis (determined by the vacuum oven) is used instead of a 15.0% moisture basis. The absorption is varied in order to produce a dough of the desired consistency, the doughs being mixed in a small Hobart mixer, equipped with two hooks, operated at No. 2 speed for three minutes. The loaves are baked in pans with low sides (top, $4\frac{1}{4}$ by $2\frac{3}{4}$ in.; bottom, $3\frac{11}{16}$ by $2\frac{1}{6}$ in.; depth, 2 in.). In all other respects the conditions laid down in the basic procedure of the A.A.C.C. are followed. Loaf volume is determined in a measuring device similar to that described by Geddes and Binnington (5) or in that described by Malloch and Cook (11). In all tests duplicate loaves were baked on different days, and if the duplicates failed to check within 20 cc. the test was repeated. Larmour, Machon, and Brockington (10) have fully described the baking routine in the laboratories collaborating in the work of the Grain Research Committee and further details may be obtained from their paper.

The loaves were judged the day following baking, the characteristics of the bread being scored on a numerical scale. Where a score less than perfect is assigned, a word or letter is appended indicating the type of fault; where no letter is given the fault is not well defined. The following factors were considered in scoring the loaves, and in assigning the key letter.

A. General Appearance

(1) Crust color – Perfect score 5.
p = color paler than ideal; d = color darker than ideal.

(2) Form – Perfect score 5.
Under this heading are included external characteristics other than crust color.
g = green, or underfermented characteristics; o = overfermented characteristics; f = flat, loaf not well risen; s = shell top.

B. Crumb Character

(1) Grain and texture – Perfect score 10.
Grain and texture are considered together, the usual significance of these terms being employed.

c = coarse — thick cell walls; o = open — large cells; close = small, round cells;

(2) Crumb color — Perfect score 10.
d = dull; g = grey; y = yellow.

In order to secure a single figure expressive of bread quality an empirical baking score is computed by summing the scores for the bread characteristics weighted in the following manner:

(Loaf volume − 400) $\times$ 0.2 =
Crust color + form =
Crumb texture $\times$ 3 =
Crumb color $\times$ 2 =
(Absorption − 60) =
Baking score ———

While the evaluation of characteristics other than volume is subject to personal judgment, the scoring in the collaborating laboratories has been made reasonably uniform by the use of reference standards and by occasional conferences where loaves differing widely in general appearance, crumb color, and texture are judged by the different operators. The work in the three laboratories is further standardized by the inclusion of a reference flour in each day's bake, the judging scores assigned to this standard being decided in advance of the baking of the experimental series. Despite a concerted attempt at standardization, it is not surprising that differences in loaf volume are obtained on the same sample in the different laboratories. The relative mean volumes for the standard flour in each laboratory, however, serve as a basis for summarizing the data. By expressing the absolute volume obtained in each laboratory as a percentage of their respective mean value for the standard, the extent of agreement can be ascertained and these percentage values averaged. The resulting figures may then

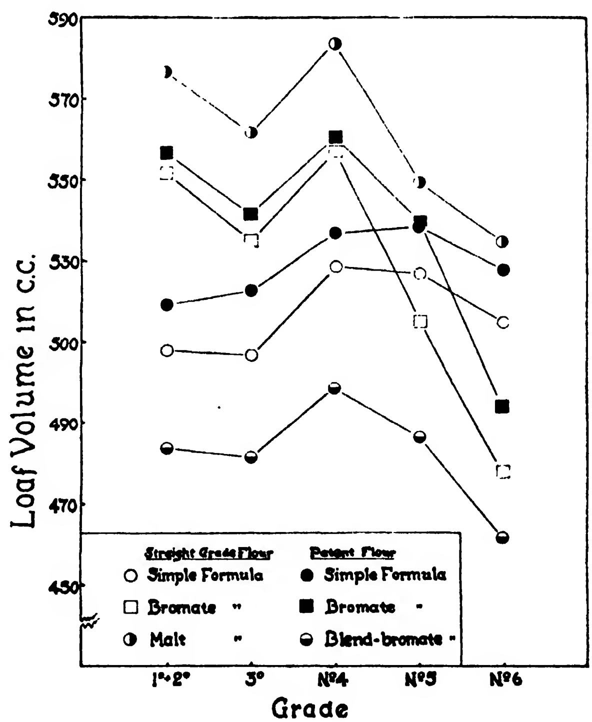

FIG. 3. *Showing the mean loaf volumes for the various grades by different baking formulas.*

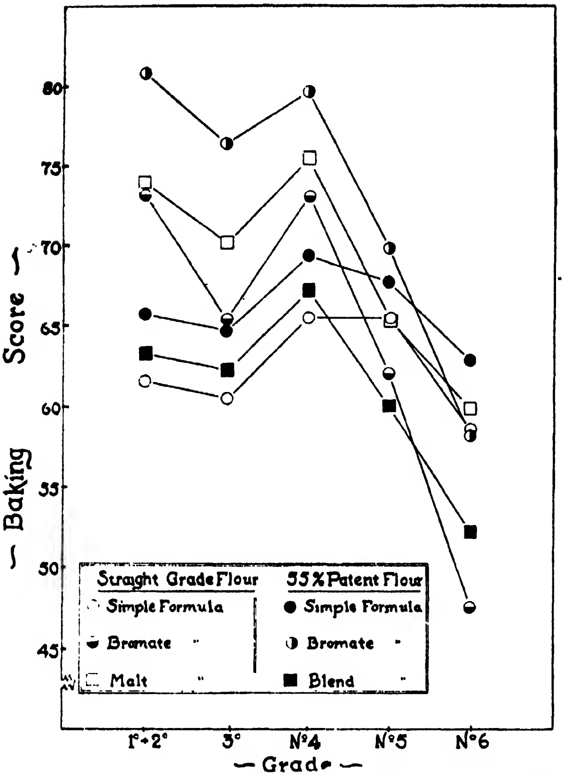

FIG. 4. *Showing the mean baking scores for the various grades by different baking formulas.*

be reconverted to loaf volume by utilizing the combined mean value obtained for the standard flour in the three laboratories, thus reducing all the results to a comparable basis.

In this study, the flours were allowed to age approximately one month before baking. The 55% patent flour was baked by the simple, bromate and blend formulas and the straight grade flour by the simple, bromate and malt formulas. The baking tests on the straight flour were all conducted in one laboratory, the patent flour was baked by the simple formula in two laboratories, while the bromate and blend formulas were applied to the patent flour in different laboratories.

The means, standard deviations and *Z* values for the baking characteristics obtained on the patent and straight grade flours are summarized in Table XIII.

The *Z* values given at the foot of each section of the table with their respective 5% points show the significance of the differences in the mean values recorded in the same column. It will be noted that the mean loaf volumes and computed baking scores recorded for the various grades are all significantly different with the exception of those obtained by the simple formula. The *Z* values for loaf volume obtained by this method on the straight flour however just exceeds the 5% point.

The mean loaf volumes and baking scores for both the patent and straight grade flours using the different baking formulas are represented graphically in Figs. 3 and 4 respectively, and illustrate the nature of the trends in these values for the various grades.

It will be observed from Fig. 3 that the baking quality as measured by loaf volume shows an increase for the simple formula from grades 1° and 2° to grade No. 5 then falls off in grade No. 6. Since the simple formula failed to reveal any significant difference in the mean loaf volumes in the instance of the patent flour and just exceeded the level of significance in the case of the straight flour, little importance can be attached to the trend indicated in the graph. If regard is paid to the trend, it reveals a progressive improvement in baking quality as measured by loaf volume as the grade of wheat decreases to No. 5.

TABLE XIII

MEANS, STANDARD DEVIATIONS AND Z VALUES FOR THE BAKING CHARACTERISTICS OBTAINED BY DIFFERENT BAKING FORMULAS

Grade	No. of samples	Absorption (13.5% M.B.) Mean %	Absorption S.D. %	General appearance Mean	General appearance S.D.	Crumb color Mean	Crumb color S.D.	Crumb texture Mean	Crumb texture S.D.	Loaf volume Mean cc.	Loaf volume S.D. cc.	Baking score Mean	Baking score S.D.
Simple Formula—Straight Grade Flour													
1° and 2°	52	64.0	1.68	7.7	0.58	6.1	1.08	5.4	0.94	507.7	33.22	61.6	10.31
3°	37	64.7	2.01	8.0	0.79	5.5	0.92	5.1	1.22	506.5	33.00	60.7	10.73
No. 4	50	65.0	1.54	8.0	0.74	5.7	1.33	5.4	1.21	528.5	41.24	65.8	13.12
No. 5	47	67.0	1.86	8.0	0.96	5.1	1.56	5.0	1.56	526.8	52.75	65.8	17.46
No. 6	36	68.3	1.70	7.5	1.21	4.0	1.16	4.4	1.49	514.6	56.65	58.7	16.88
Z		1.8903		0.5191		1.3953		0.6911		0.4641		0.3853	
5% Pt.		0.4404		0.4404		0.4404		0.4404		0.4404		0.4404	
Simple Formula—55% Patent Flour													
1° and 2°	52	64.5	0.99	7.1	0.86	6.4	1.10	5.9	1.26	519.0	28.11	65.9	9.25
3°	38	65.1	1.55	7.1	1.18	5.9	1.02	5.4	1.20	522.6	29.61	64.9	9.35
No. 4	51	65.1	1.29	7.6	1.05	6.2	1.09	5.7	1.18	536.6	37.00	69.5	10.42
No. 5	48	66.1	1.74	8.0	1.19	5.2	1.59	5.1	1.71	538.5	55.32	68.0	16.65
No. 6	39	67.9	2.10	8.2	1.26	4.1	1.58	4.4	1.66	527.7	58.81	63.3	18.08
Z		1.7219		1.0836		1.5514		0.9780		0 3055		0.2255	
5% pt.		0.4403		0.4403		0.4403		0.4403		0.4403		0.4403	
Bromate Formula—Straight Grade Flour													
1° and 2°	50	63.9	1.62	8.0	0.89	6.3	1.00	6.1	1.26	551.9	56.24	73.2	16.17
3°	36	64.6	2.04	7.9	1.04	5.7	1.12	5.7	1.41	534.2	59.50	65.5	17.23
No. 4	49	65.0	1.60	7.9	0.98	6.1	1.12	5.7	1.25	556.7	63.09	73.1	17.29
No. 5	43	66.8	1.77	7.2	1.17	5.0	1.31	4.4	1.18	515.8	76.67	62.2	19.12
No. 6	33	68.1	1.66	6.5	1.05	3.6	1.06	3.7	1.21	477.3	70.95	47.3	18.79
Z		1.8093		1.3125		1.7732		1.6152		1.1624		1.2976	
5% pt.		0.4408		0.4408		0.4408		0.4408		0.4408		0.4408	
Bromate Formula—55% Patent Flour													
1° and 2°	48	65.0	1.65	7.1	1.37	7.2	1.95	8.0	1.36	556.4	59.56	80.9	18.18
3°	34	65.1	2.09	7.3	1.58	6.7	1.75	7.1	2.36	541.6	62.33	76.3	22.47
No. 4	43	64.9	1.81	7.3	1.69	7.2	1.67	7.6	1.76	560.2	65.09	79.7	20.61
No. 5	39	66.0	1.84	7.2	1.91	5.3	2.31	6.1	2.46	539.6	76.28	69.8	25.02
No. 6	34	68.1	2.38	6.5	2.01	3.9	2.35	4.9	2.49	494.0	70 92	57.8	22.46
Z		1.4237		0.1669		1.4608		1.2974		0.9277		1.5485	
5% pt.		0.4414		0.4414		0.4414		0.4414		0.4414		0.4414	
Blend Formula—55% Patent Flour													
1° and 2°	52	65.0	1.05	8.4	0.94	6.7	1.01	6.4	1.00	483.7	33.18	63.3	10.29
3°	38	65.4	1.11	8.5	1.09	6.5	1.02	6.4	1.16	481.6	36.88	62.1	12.20
No. 4	51	65.4	0.91	8.7	0.79	6.7	1.02	6.5	0.98	498.7	36.17	67.2	11.79
No. 5	48	67.1	2.52	8.6	1.26	5.4	1.47	5.4	1.49	486.4	55.10	60.0	16.71
No. 6	39	69.4	2.36	8.2	1.39	4.2	1.42	4.6	1.60	461.7	52.13	52.2	16.96
Z		1.9322		0.1578		1.7914		1.4223		0.6967		0.9534	
5% Pt.		0.4403		0.4403		0.4403		0.4403		0.4403		0.4403	
Malt Formula—Straight Grade Flour													
1° and 2°	52	62.9	1.60	7.8	0.50	5.9	1.03	5.3	1.22	576.7	47.63	73.8	12.37
3°	38	63.7	2.07	7.7	0.76	5.6	1.16	5.2	1.24	561.5	42.46	70.3	13.37
No. 4	51	64.0	1.70	7.7	0.79	5.8	1.35	5.2	1.13	583.3	45.03	75.8	13.34
No. 5	48	66.0	1.88	7.2	1.16	4.6	1.31	4.2	1.27	549.1	68.64	65.5	19.41
No. 6	39	67.4	1.61	6.6	1.05	4.0	1.34	3.8	1.20	534.5	66.74	60.1	18.33
Z		1.9305		1.3002		1.4814		1.2962		0.8280		0.9968	
5% Pt.		0.4403		0.4403		0.4403		0.4403		0.4403		0.4403	

This result is entirely incongruous with the commercial evaluation of the grades and confirms the observations previously published by Larmour and MacLeod (9) in regard to the value of the simple formula. It should be added that the data for the straight grade flour obtained in one laboratory are in close relative agreement with the average results of the two laboratories which tested the patent flour.

It should be pointed out here that the simple formula as applied to experimentally milled flours is not comparable to commercial practice. Experimental flours are unbleached and usually receive little or no aging before being submitted to the baking test. Commercial flours are normally aged for a much longer period, and frequently bleached with agents which act also as flour improvers, and in addition may have an improver added in the commercial bakeshop.

The trends in loaf volume by all the other baking formulas are highly significant and are quite similar. In each instance the curve falls from 1° and 2° to 3°, rises sharply to a maximum value in grade No. 4 and then declines abruptly for grades No. 5 and No. 6. It is of interest to note the close parallelism in the two curves for the results by the bromate formula. The same general trends are evident for the baking scores as will be seen from the graph.

Considering now the trends in the means for the individual baking characteristics, absorption, general appearance, crumb color, and crumb texture given in Table XIII, these are all significant with the exception of general appearance as recorded for loaves baked by the bromate and blend formulas.

Absorption increases markedly from grades 1° and 2° to grade No. 6 for all the baking formulas, the absolute values being somewhat lower for the malt formula. The doughs containing malt were slightly sticky and showed a tendency to "slacken" during fermentation, and for this reason less water was used in mixing. High absorption is a characteristic of flours milled from frosted wheat and in individual cases ran as high as 75%.

The means for general appearance, crumb color, and crumb texture, show, with the exception of No. 4, a fairly progressive trend towards lower values as the grade decreases. As the type of fault for which the loaves were scored down varied considerably within each grade it was not possible to affix a key letter describing the various loaf characteristics in the table of means.

In a general way, the external appearance of the loaves was quite similar until grade No. 6 was reached when the crust color possessed a characteristic dull hue. The crumb texture was quite similar for grades 1° and 2° to No. 4, that of grade No. 4 being equal on the average or superior to the scores recorded for 3°. The textures of the loaves baked from flours milled from No. 5 and No. 6 wheats however were distinctly inferior, being "open" and possessing thick cell walls. The crumb colors recorded for grade No. 4 were superior to those for 3°, varying from "creamy" to "creamy yellow". The predominating crumb color in the case of grade No. 5 was a "dull creamy" and in grade No. 6, "dull grey".

The grade No. 4 wheats thus yielded, on the average, flours which possessed superior baking quality to 3° as measured not only by loaf volume, but also

by crumb color and crumb texture. Although the present grading system gives a satisfactory differentiation of baking quality when the contract grades are considered as one group and the commercial grades as another, the transition from the one to the other, that is from 3° to No. 4, is not satisfactory. It is, therefore, not surprising that, in the instance of the 1928 crop, there was a frequent preference among British millers for "fours" rather than "threes" on the basis of quality independent of price. Since, in the 1930 Amendments to the Canada Grain Act, the definition of 3° was changed to require it to contain 25% hard vitreous kernels this anomaly is not likely to recur.

A reasonable explanation suggests itself for the anomalous relationship between 3° and No. 4. Samples which are graded into the contract grades are composed principally of sound kernels, the distinction between the grades being made chiefly on the basis of percentage of starchy kernels. Three Northern, therefore, represents the poorest portion of the sound samples. In a frosted wheat crop, such as that of 1928, the differentiation of the commercial grades is made on the basis of percentage of visible damage, No. 4 being, of course, the least damaged of the group. Had they not been frozen, it may be assumed that the samples grading No. 4 would have been divided among the contract grades. Thus, in effect, the average quality of the samples with the highest percentages of starchy kernels is being compared with the average quality of samples ranging from entirely vitreous to entirely starchy but damaged in appearance by frost and the presence of immature and green kernels. Although the latter wheats were damaged in appearance, these samples in reality had a higher baking quality, and it must be concluded that the actual damage due to the presence of frosted, immature and green kernels was not sufficient to mask the inherent higher quality of the samples in grade No. 4. The foregoing explanation does not completely explain the results since the grade No. 4 wheats showed superior strength not only to those of 3°, but also to the averages of 1° and 2°. It must be recalled, however, that only nine samples grading 1° were included in this study. If a sufficient number of representative samples for this grade had been collected, it would have been possible to consider the results for 1° separately, and would serve as a basis for indicating the probable correctness of the suggested explanation. The further possibility of improvement in the baking quality of No. 4 wheat as a direct result of the presence of a limited number of frosted kernels is now under investigation.

Relation Between Loaf Volumes Determined by Different Baking Formulas

In order to measure the extent of the relationship between the loaf volumes obtained by the various baking formulas employed, the simple correlations given in Table XIV have been calculated. The first two sets of correlations given in the table are of a high order and indicate that the straight grade and patent flours give the same relative results when comparisons are made by either the simple or by the bromate formulas.

Correlations within grades were determined by Z transformation (3) and were approximately the same as for the entire population. Correlations between the mean values for the different grades were calculated for r_{ac} and r and

were + .952 and + .907 respectively. These correlations show that all of the samples may be looked upon as a uniform population, about the same relation existing between as within the grades.

TABLE XIV

THE RELATION BETWEEN LOAF VOLUMES OBTAINED BY DIFFERENT BAKING FORMULAS

	r	Simple correlation coefficients—wheat grade						
		1° and 2°	3°	No. 4	No. 5	No. 6	Within grades	All grades
Simple formula straight flour (a)× simple formula patent flour (c)	r_{ac}	+0.754	+0.821	+0.672	+0.842	+0.967	+0.839	+0.839
Bromate formula straight flour (e)× bromate formula patent flour (g)	r_{eg}	+ .681	+ .818	+ .851	+ .884	+ .821	+ .819	+ .837
Simple formula straight flour (a)× blend formula patent flour (b)	r_{ab}	+ .429	+ .750	+ .632	+ .805	+ .771	+ .686	+ .696
Simple formula patent flour (c)× blend formula patent flour (b)	r_{cb}	+ .574	+ .669	+ .755	+ .818	+ .893	+ .758	+ .762
Bromate formula straight flour (e)× blend formula patent flour (b)	r_{eb}	+ .665	+ .783	+ .774	+ .884	+ .829	+ .795	+ .798
Bromate formula patent flour (g)× blend formula patent flour (b)	r_{gb}	+ .580	+ .773	+ .695	+ .869	+ .882	+ .774	+ .785
Approximate value of r at 5% pt.		.27	.32	.28	.28	.31	.13	.13

In this study, baking tests were conducted on both a long patent (here called straight) and a short patent in view of the possibility that they would not give the same relative results on wheats containing a high percentage of frosted kernels if the frost damage were chiefly external in character. The correlations suggest that in experimental determinations of wheat strength it makes little difference whether a patent or a straight flour is milled. This result is of considerable importance in a cereal laboratory as the milling of a straight yields more flour from a given quantity of wheat, thus permitting a more thorough investigation of the baking behavior.

The results by the bromate formulas are highly correlated with those by the blend, while the correlations between the data for the simple and blend are not of such a high order of magnitude. In the latter case in particular, there is some indication of a trend in the correlations within grades towards higher values as the grade decreases.

Response to Differential Baking Tests

Information of practical and scientific interest may be gained by a study of the differences between the results obtained by the different baking formulas. Table XV shows the mean responses in loaf volume and baking scores to potassium bromate, to malt and to blending, using the results by the simple formula with the same kind of flour as a base. The significant Z values show that the trends toward lower mean responses for each of the differential baking tests as the grade of wheat decreases are highly significant. The graph

presented in Fig. 5 showing the mean responses in loaf volume illustrates the nature of these trends more clearly. The curves for the two bromate responses parallel each other and indicate the close relative agreement not only between the two laboratories conducting the tests, but also in the information given by the patent and straight grade flours. The mean response to bromate and to malt of the flours milled from No. 4 wheats is of the same order of magnitude as for 3°.

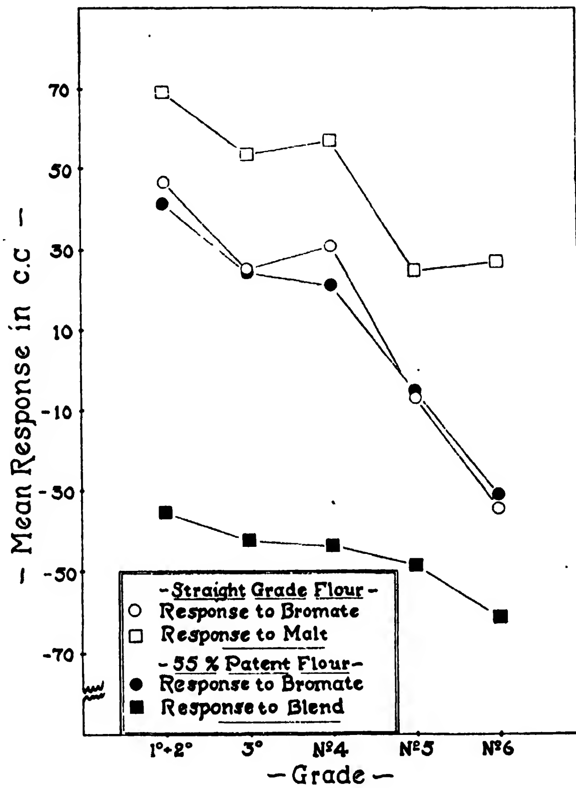

FIG. 5. *Showing the mean responses in loaf volume to differential baking tests.*

The literature dealing with the bromate differential test has been reviewed by Larmour and MacLeod (8) and need not be detailed here. The precise action of potassium bromate is not known but it influences gas retention by some effect, direct or indirect, on the protein, but has no measurable influence on gas production, and the magnitude of the response is associated in part with protein quantity and in part with other factors usually designated as protein "quality", as yet little understood. Flours giving a strong positive response to the bromate differential test are regarded commercially as possessing reserve strength and a high fermentation tolerance. On the other hand, flours showing no appreciable response usually show high baking quality by the simple formula and have little or no reserve strength, while a negative response to bromate indicates weakness and a lack of ability to produce good bread unless handled under ideal conditions.

A high response to malt indicates that the gas production is not sufficient to make full use of the gas-retaining capacity of the dough, while a low response indicates that the gas production is already adequate or that gas retention is poor. The response to the blend formula is obviously a measure of the reserve strength or blending capacity of the flours and is especially important in the testing of quality of Western Canadian wheat which is largely sold for export for blending purposes.

TABLE XV

MEAN RESPONSES IN LOAF VOLUME AND BAKING SCORE TO FLOUR IMPROVERS OR BLENDING, STANDARD DEVIATIONS AND Z VALUES

Grade	Loaf volume in cc.								Baking score							
	Response to bromate				Response malt		Response blend		Response to bromate				Response malt		Response blend	
	Straight grade flour		55% patent flour		Straight grade flour		55% patent flour		Straight grade flour		55% patent flour		Straight grade flour		55% patent flour	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
1° and 2°	46.3	45.96	41.1	56.92	69.0	32.88	−35.6	29.68	11.9	15.62	16.6	16.11	12.4	8.67	−1.7	9.12
3°	24.8	50.64	24.0	52.86	53.6	33.42	−42.5	28.80	3.7	15.50	14.6	17.71	9.9	8.62	−3.3	8.29
No. 4	30.2	52.92	20.6	46.38	56.4	34.26	−43.9	33.42	8.0	15.44	11.4	15.19	9.6	9.03	−3.1	11.39
No. 5	− 7.3	48.60	− 5.2	40.86	24.1	39.06	−48.6	33.60	− 5.1	15.11	1.5	13.03	0.1	9.44	−6.3	11.22
No. 6	−34.3	39.48	−31.4	45.30	26.2	36.12	−61.4	37.98	−11.6	12.76	− 5.6	13.04	1.3	8.44	−10.9	11.39
Z	1.4157		1.2669		1.3123		0.6382		1.3456		1.3307		1.4274		0.8217	
1% pt.	.6139		.6146		.6130		.6186		.6139		.6146		.6130		.6126	

On the basis of the above discussion the significant decreases in the mean responses to the differential tests from grade to grade represent inherent differences in baking behavior. Flours milled from the lower grade wheats, aside from their inferior baking quality, must be regarded as being less desirable commercially because of lower blending strength and the indications of poor fermentation tolerance. In general, the grading system satisfactorily differentiates wheats in these respects although No. 4 is somewhat out of line, giving responses to malt and bromate of the same order as 3°. The standard deviations given in Table XV, however, show that the variability in the responses within each grade is very high.

Since the mean protein content is practically the same for the various grades, the differences in mean response must be attributed largely to differences in protein quality, and it is of practical and scientific interest to evaluate the relative importance of quantity and quality. For this purpose simple, partial, and multiple correlations for some of the responses have been calculated for all grades combined. The results are given in Table XVI. The simple correlations between wheat protein and response to bromate, malt, and blending, indicate that the magnitude of the responses are in part associated with the quality of protein present in the flour.

Taking the response to bromate as representative, the second order partial correlations and the multiple given in Table XVII have been calculated in order to determine the relation betwen protein content, percentage of sound kernels and bromate response. The correlation $r_{6B.12}$ gives the relation between per cent wheat protein and bromate response when the percentage of total sound and bran frosted kernels are held constant. The correlation $r_{1B.62}$ shows the relation between bromate response and percentage sound kernels with protein and bran frost constant. Since "heavy damage" (the sum of heavily frosted, immature and green kernels) designated by key figure 7 is the complement of total sound and bran frost, it is obvious that $r_{7B.62}$ = approximately $-.407$.

TABLE XVI

CORRELATIONS INVOLVING RESPONSES TO DIFFERENTIAL BAKING TESTS

	Simple correlation coefficients					Second order partial correlation coefficients for response to bromate (str. flour)
	Wheat protein (6)	Total sound (1)	Bromate response (str. flour) (B)	Malt response	Blend response	
Wheat protein (6)			+ .319	+ .316	+ .148	$r_{6B.12}$ = + .469
Total sound (1)	− .031		+ .527			$r_{1B.62}$ = + .407
Bran frost (2)	+ .183	− .785	− .344			
	At 5% pt. $r = .138$					At 5% pt. $r = .139$

MULTIPLE CORRELATION: $R_{B(62)} = .625$; 5% pt. = .240

It seems logical to presume that the inherent protein quality of frosted, green and immature kernels would be inferior to that of sound kernels and if this assumption is made, the data show that bromate response is associated, in part at least, with the quantity and quality of the protein. The multiple showing the combined effect of protein content and percentage sound kernels is not sufficiently high to suggest that these are the only factors involved in the response to bromate.

TABLE XVII

REGRESSION OF VARIABILITY IN BAKING QUALITY ON MEAN PERCENTAGE OF TOTAL SOUND KERNELS IN EACH GRADE

Grade	Mean total sound kernels %	Variability of: Loaf volume bromate formula straight flour σ_e	Loaf volume blend formula patent flour σ_b	Baking score blend formula patent flour σ_L
1° and 2°	85.5	56.24	33.18	10.29
3°	74.2	59.50	36.88	12.20
No. 4	58.6	63.09	36.17	11.79
No. 5	30.8	76.67	55.10	16.71
No. 6	18.6	70.95	52.13	16.96
Regression coefficient	b	−0.285	−0.339	−0.105
	t	4.35	4.35	6.03
	5% Pt.	3.18	3.18	3.18

Variability in Baking Quality Within Grades

The variability of the baking characteristics within each grade is recorded in Table XIII in terms of the standard deviation. Taking loaf volume and baking score as representative of baking quality it will be noted that there is a trend towards increased variability in the lower grades. The significance of this regression of variability on grade has been determined using the variability in loaf volume and baking score by the blend formula, and loaf volume by the bromate formula (straight flour) as typical examples. In order to determine the significance of the regression, the mean percentage of sound kernels has been taken as the best available measure of grade since the grade numbers themselves do not represent numerical values. The regression coefficients are given in Table XVII with their respective t values and 5% points. The trend towards higher variability in the baking quality of the lower grades is highly significant. It will be observed, however, that the variability in grade No. 4 is of the same order as that in the contract grades.

The Relation Between Wheat Protein and Baking Quality

The relation between wheat protein and baking quality as measured by loaf volume for each baking formula is given in Table XVIII for the individual grades and for all grades combined. The correlations are not of a high order,

those for the bromate, blend and malt formulas being of greater magnitude than the corresponding values for the simple formula. The significance of the difference in the correlations by the various baking methods has been determined for the entire series. This is expressed by the ratio of the difference in Z values for each pair of correlation coefficients $(Z_1 - Z_2)$ to its standard deviation $\sigma_{(Z_1-Z_2)}$. The results given in Table XIX show that there is no significant difference in the correlations for the straight grade and patent flour by either the simple or bromate formula. However, those between protein and loaf volume by the malt and bromate formulas are significant, confirming the observations of Larmour (6, 7) in this connection.

TABLE XVIII

SIMPLE CORRELATIONS BETWEEN PROTEIN AND LOAF VOLUME OBTAINED ON 55% PATENT AND STRAIGHT GRADE FLOUR BY DIFFERENT BAKING FORMULAS

Grade	Simple formula		Bromate formula		Blend formula	Malt formula	Approx. value of r at 5% pt.
	Straight flour (a) r_{6a}	55% Patent flour (c) r_{6c}	Straight flour (e) r_{6e}	55% Patent flour (g) r_{6g}	55% Patent flour (k) r_{6k}	Straight flour (m) r_{6m}	
1° and 2°	+ .469	+ .358	+ .599	+ .534	+ .310	+ .692	.27
3°	+ .509	+ .354	+ .674	+ .604	+ .634	+ .695	.32
No. 4	+ .304	+ .332	+ .820	+ .678	+ .572	+ .661	.28
No. 5	+ .620	+ .597	+ .671	+ .707	+ .670	+ 746	.28
No. 6	+ .541	+ .581	+ .621	+ .549	+ .666	+ .598	.31
All grades	+ 460	+ .425	+ 605	+ .571	+ 526	+ 627	.13

The analysis of covariance for wheat protein and various measures of baking quality is given in Table XX and shows that the correlation within grades is somewhat higher than the total. The correlation between grades, which

TABLE XIX

SIGNIFICANCE OF THE DIFFERENCE BETWEEN CORRELATIONS FOR PROTEIN WITH LOAF VOLUME (ALL GRADES COMBINED) OBTAINED BY DIFFERENT BAKING FORMULAS

	Simple correlations		$\frac{Z_1-Z_2}{\sigma(Z_1-Z_2)}$	5% Point
	Straight flour	Patent flour		
Simple formula Bromate formula	+ .460 + .605	+ .425 + .571	0.34 0.56	1.64 1.64
Simple formula Bromate formula	+ .460 + .605		2.17	1.64
Simple formula Bromate formula		+ .425 + .571	2.05	1.64
Simple formula Malt formula	+ .460 + .627		2.53	1.64
Simple formula Blend formula		+ .425 + .526	1.38	1.64

expresses the relation between the mean protein and mean baking quality for different grades, is insignificant. To illustrate this more clearly the grade means for wheat protein and loaf volumes by the bromate formula straight flour are given at the foot of Table XX. The mean protein content for the various grades is practically constant, yet there is a wide range in the mean loaf volumes. The variation in mean loaf volume with an approximately constant mean protein is a reflection of the differences in the average protein quality and other factors affecting strength of the various grades.

This is very important from the standpoint of grain grading. Since all grades in 1928 had approximately the same mean protein content, this factor obviously could not have been used to advantage as a basis for differentiating between the grades, but rather as an index of quality within each grade. The variability in baking within the grades is apparently due in large part to the variations in protein content.

TABLE XX

ANALYSES OF COVARIANCE FOR WHEAT PROTEIN AND VARIOUS MEASURES OF BAKING QUALITY

Simple correlation coefficients for	Wheat protein and loaf volume: Simple formula, 55% patent flour		Wheat protein and loaf volume: Bromate formula, straight flour		Wheat protein and loaf volume: Blend formula, 55% patent flour		Wheat protein and computed baking score for blend formula		5% Points	Degrees of freedom
	r_{6c}	t	r_{6e}	t	r_{6k}	t	r_{6L}	t		
Total, all grades	+ .425	7.1	+ .605	11.4	+ .526	9.3	+ .536	9.5	1.96	226
Between grades	− .043	0.1	− .014	0.0	− .274	0.5	− .230	0.4	3.18	3
Within grades	+ .440	7.2	+ .674	13.5	+ .561	10.0	+ .584	10.6	1.96	218

Grade	1° and 2°	3°	No. 4	No. 5	No. 6
Mean wheat protein, %	12.2	11.8	12.1	12.0	12.3
Mean L. V. (cc), bromate formula (straight flour)	552	534	557	516	477

The Relation Between Protein Content, Classes of Visible Damage and Baking Quality

The relation between protein content, classes of visible damage, and baking quality is expressed by the simple, partial and multiple correlation coefficients for the entire series in Table XXI. In these calculations, loaf volume by the bromate (patent flour), blend, and malt formulas, and baking score by the blend formula have been taken as representative of baking quality. The simple correlations involving wheat protein and the various classes of kernels show, as might be surmised from a knowledge of the biochemical changes in the maturation of the wheat kernel, that the protein content of wheat is not

TABLE XXI

SIMPLE, PARTIAL AND MULTIPLE CORRELATIONS SHOWING THE RELATION BETWEEN PROTEIN CONTENT, CLASSES OF VISIBLE DAMAGE AND VARIOUS MEASURES OF BAKING QUALITY

	Simple correlation coefficients					Fourth order partial correlation coefficients							
	Wheat protein (6)	L.V. pat. bromate (g)	L.V. pat. blend (k)	L.V. str. malt (m)	Bak. score pat. blend (L)	Loaf volume 55% patent bromate formula (g)		Loaf volume 55% patent blend formula (k)		Loaf volume straight malt formula (m)		Baking score 55% patent blend formula (L)	
Total sound (1)	− .016	+ .268	+ .158	+ .258	+ .263								
Bran frost (2)	− .000	− .176	− 149	− .243	− .224	$r_{2g.3456}$	− 097	$r_{2k.3456}$	− .122	$r_{2m\ 3456}$	− .258	$r_{2L.3456}$	− .193
Heavy frost (3)	+ .135	− .167	− .033	− .036	− 067	$r_{3g.2456}$	− .245	$r_{3k.2456}$	− 074	$r_{3m\ 2456}$	− .076	$r_{3L.2456}$	− .100
Immature (4)	− .172	− .195	− .161	− .266	− .248	$r_{4g\ 2356}$	− .110	$r_{4k.2356}$	− 069	$r_{4m\ 2356}$	− .196	$r_{4L.2356}$	− .169
Green (5)	+ .056	− .123	− .074	− .091	− 132	$r_{5g\ 2346}$	− .103	$r_{5k.2346}$	− .057	$r_{5m\ 2346}$	− .037	$r_{5L.2346}$	− .087
Wheat protein (6)		+ .571	+ .526	+ .627	+ 536	$r_{6g.2345}$	+ .599	$r_{6k.2345}$	+ .526	$r_{6m.2345}$	+ .635	$r_{6L\ 2345}$	+ .543
	At 5% pt. $r = .132$					At 5% pt. $r = .132$							

MULTIPLE CORRELATIONS

$R_{g(23456)} = .643$ $R_{k(23456)} = .557$ $R_{m(23456)} = .692$

$R_{L(23456)} = .609$

At 5% pt. $R = .320$

related in any degree to the individual forms of visible damage. The simple correlations reveal that the best single index of baking quality is protein content, the next being the percentage of total sound kernels, which, however, is a much less reliable measure. The simple correlations between the various classes of damage are low and for the most part insignificant, and indicate that individually they are of little or no value as grading factors.

The fourth order partial correlations show the relation between protein, percentage bran frost, heavy frost, immature, and green kernels respectively on baking quality, when each factor varies alone. It will be observed that none of the individual forms of damage have any appreciable effect on baking quality when the other classes of damage and protein are held constant. In view of these correlations it would seem that in a series of samples differing inherently in quality, apart from damage, the assumption that baking quality can be estimated by the amounts of individual kinds of damage is based on a false analogy to the depreciation in quality found when varying percentages of different forms of damaged kernels are added to samples of a single lot of sound wheat. The relation between protein content and the various measures of baking quality when all forms of damage or in other words, the percentage of total sound kernels, are held constant is, however, quite significant.

It is apparent from the multiple correlations involving the same factors, the largest of which is 0.69, that all the variables have not been accounted for. This is probably due in part to variations in the inherent quality of the sound kernels.

The data show that in so far as baking quality is concerned, the various forms of damage do not require individual consideration in grading and that protein content and percentage of sound kernels are the best single indices of quality.

These conclusions are based on the results for the entire series and there seemed a possibility that the relations of protein and the different classes of visible damage to baking quality might be quite different for the lower grades. Fourth order partials were therefore calculated for grades No. 5 and No. 6 using the baking score for the blend formula as a measure of baking quality. The results given in Table XXII again show that the individual forms of damage are slight and approximately equally correlated with baking quality when varied alone, and that protein content is the most reliable single measure of the baking quality of low grade wheats. The general conclusion is hence the same, whether we consider the entire series collectively or the low grade wheats as a group, that protein content, and the percentage of sound kernels are the best single measures of baking quality.

TABLE XXII

FOURTH ORDER PARTIAL CORRELATION COEFFICIENTS SHOWING THE RELATION BETWEEN PROTEIN CONTENT, CLASSES OF VISIBLE DAMAGE AND BAKING SCORE (BLEND FORMULA) FOR GRADES NOS. 5 AND 6 COMBINED

L = Baking score, blend formula	
2 = % Bran frost	$r_{L2.3456} = -.305$
3 = % Heavy frost	$r_{L3.2456} = -.302$
4 = % Immature	$r_{L4.2356} = -.393$
5 = % Green	$r_{L5.2346} = -.207$
6 = % Wheat protein	$r_{L6.2345} = +.638$

At 5% pt. $r = .205$

Discussion

The results of this study based on representative samples of the 1928 wheat crop of Western Canada show that the grading system in use that year gives a correct indication of the average relative milling and baking quality of the grades except in the case of No. 4. Although there was a progressive decrease in mean flour yield as the grade decreased, the average baking quality of No. 4 wheat was distinctly superior to that of 3°, and in fact even to grades 1° and 2° combined. It must be recalled that only nine samples grading 1° were included in this study. Wheat quality depends on both the yield and strength of flour and it seems probable that, from the viewpoint of the European miller, the greater strength of No. 4 is more than sufficient to offset its lower flour yield. This anomaly in the relative quality of these grades is not now likely to occur in view of the amendment to the Canada Grain Act in 1930 requiring 3° to contain 25% of hard red vitreous kernels.

In the Canadian grading system, weight per measured bushel and percentage of hard red vitreous kernels are the chief factors used in assigning the statutory grade, while the relative amounts of the various classes of damage present in the samples are paramount in deciding the commercial grades of frosted wheat. The progressive change in the character of the damage in the different commercial grades indicates that the system is applied in a very efficient manner by the Grain Inspection Division.

The data presented here strongly indicate that weight per measured bushel is as valuable an index of flour yield in the commercial as in the statutory grades. Individually, bran frosted, heavily frosted, immature and green kernels do not show a close relation to wheat quality and hence an estimation of the amount of any one particular form of damage is of little value as a grading factor. Collectively, however, they exert an appreciable influence on both milling and baking quality. This implies that the percentage of total damage, or conversely the percentage of sound kernels present, forms the most logical basis for the grading of frosted wheat into the commercial grades.

The adoption of weight per bushel and the percentage of sound kernels as the chief factors in assigning the commercial grades to frosted wheat is an extension of the principles employed in the statutory grades and would thus lead to a unification of the entire grading system. The various classes of sound kernels were grouped together in this preliminary study, in order to simplify the statistical treatment and discussion of the data. In most instances, the percentage of piebald and starchy kernels was not great and there was a close relation between the percentages of total sound and vitreous kernels. Several calculations not reported here were made in which the percentage of vitreous kernels was substituted for "total sound" and the correlations with milling and baking quality were slightly higher. For the sake of uniformity, it would seem desirable to use the percentage of vitreous kernels as a grading factor throughout.

With the exception of the anomalous relationship in the strength of 3° and No. 4 wheats, the grading system gives a satisfactory indication of the

average relative quality of the grades. The variability in flour yield and baking quality within each grade, particularly in the instance of the commercial grades, however, is high, and thus the system fails to give an accurate evaluation of the quality of the individual samples. The high variability within grades is of particular importance to the producer who is interested in the grading of his own comparatively small lot of wheat and the securing of the highest grade to which its quality will entitle it.

While variations in the percentage of sound kernels are in part responsible for the differences in quality within each grade the protein content of the wheat is a much more important factor. The data clearly show that within each grade protein is the most reliable single index of strength, this statement applying equally to the higher and the lower grades. There is, however, no relationship between the mean protein content of the different grades and the average baking quality, implying that, in general, the grades are clearly differentiated on the basis of protein "quality". This is indicated by the decrease in mean response to the differential baking tests with lowering wheat grade.

Since the magnitude of the response is in part related to the percentage of total damage present and the percentage of protein is not related to the classes of kernels, protein content of wheat could not logically be used as a primary grading factor. The ideal grading system, apart from an actual determination of milling and baking quality, would appear to be a primary subdivision into wide groups on the basis of weight per measured bushel and the percentage of hard red vitreous kernels, followed by subclassifications within each group on the basis of protein content.

Since experimental evidence has been accumulating in recent years confirming the conclusion that the protein content of wheat is a good indication of its baking strength and value for blending with weaker wheats, an inquiry has recently been conducted by Newton (12) regarding the feasibility of using protein content as a factor in grading and marketing Canadian wheat. In general, the European purchasers of Canadian wheat discounted the value of the protein test because it indicated quantity rather than quality; the outstanding requirement of the European trade being constancy of grade qualities. In view of the general satisfaction of the European trade with the present grading system and the practical difficulties which would be encountered in the grading, storage and transportation of the crop if protein were utilized as a grading factor, it was not considered feasible at the present time.

The data presented here indicate that constancy of grade qualities in relation to baking behavior cannot be expected with wide variations in protein content, but some improvement in this respect may be effected by reducing variations in quality which depend in part on the percentage of sound kernels present. The recent revision of the Canada Grain Act which raised the percentage of hard red vitreous kernels in the different grades may be expected to bring about a greater uniformity in grade characteristics and the changes made are in line with the results of this study. Owing to the variable nature of wheat and the complexity of the characteristics to be evaluated, it is doubtful whether

it will ever be possible to devise a grading system which will indicate accurately the value of every sample and at the same time be of practical application.

In conclusion, the authors wish to point out that the experimental material on which this study was based was collected in only one crop year and is therefore representative of only one general class of frost damage. In that year, 1928, frost occurred when the wheat was nearly mature, and extended into areas which do not usually produce frosted wheat. The annual surveys now in progress may be expected to show to what extent the various forms of frost damage are correlated with wheat quality in other years.

Acknowledgments

The authors are indebted to the Canadian Co-operative Wheat Producers Limited who extended their facilities in securing the samples and to the Board of Grain Commissioners for the work done by the Inspection Branch and the Grain Research Laboratory. They are deeply indebted to Dr. C. H. Goulden, Cerealist, Dominion Rust Research Laboratory, Winnipeg, for his counsel and advice in connection with the statistical reduction of the data. They also wish to acknowledge the aid of the technical assistants in the three collaborating laboratories.

References

1. American Association of Cereal Chemists. Methods for the analysis of cereals and cereal products. Lancaster Press Inc., Lancaster, Pa. 1928.
2. Blish, M. J. Cereal Chem. 5: 158-161. 1928.
3. Fisher, R. A. Statistical methods for research workers. 3rd ed., Oliver and Boyd, London. 1930.
4. Geddes, W. F. Can. J. Research, 1: 528-558. 1929.
5. Geddes, W. F. and Binnington, D. S. Cereal Chem. 5: 215-220. 1928.
6. Larmour, R. K. Cereal Chem. 7: 35-48. 1930.
7. Larmour, R. K. Cereal Chem. 8: 179-189. 1931.
8. Larmour, R. K. and MacLeod, A. G. Sci. Agr. 9: 477-490. 1929.
9. Larmour, R. K. and MacLeod, A. G. Sci. Agr. 10: 1-22. 1929.
10. Larmour, R. K., Machon, F. D. and Brockington, S. F. Cereal Chem. 8: 233-241. 1931.
11. Malloch, J. G. and Cook, W. H. Cereal Chem. 7: 307-310. 1930.
12. Newton, R. Bulletin No. 14. National Research Council of Canada. 1930.

THE EFFECT OF STORAGE AT VARIOUS MOISTURE CONTENTS ON BAKING QUALITY OF MARQUIS WHEAT[1]

By R. K. Larmour[2]

Abstract

Marquis wheat, in 5-lb. containers, at moisture contents ranging from 10 to 22%, was stored at 21°C. and at outside winter temperature for periods of one and four months. With the exception of samples having 16, 18, 20, and 22% moisture, stored at 21°C. for four months, no evidence either of improvement or deterioration of baking quality was observed. The exceptions noted were lower in quality due principally to mustiness of the wheat.

Introduction

In some seasons in Western Canada harvesting is followed by a period of wet weather that delays threshing very considerably and results in appearance on the market of tough and damp wheat. Tough wheat contains over 14.4% and not more than 17.0%, and damp wheat contains more than 17.0%, moisture. Wheat falling in either of these categories cannot be graded "straight" but is designated "no grade". The proportion of this "no grade" wheat is very large in some years. For the crops produced in 1925, 1926, and 1927, the percentages of this class of wheat were 28.6, 50.8, and 44.7 respectively. The limits set for tough wheat are based upon observations of the moisture content at which wheat may be stored without danger of damage from heating. While the main problem with this class of wheat has been to ascertain exactly the optimum safe condition for drying, there must also be considered the question whether or not the wetting and subsequent storage, under conditions that preclude heating, have any effect on the baking quality of the wheat. Wheat that has been tough or damp and subsequently dried to below 14.4% moisture often exhibits a marked change in appearance, the color becoming dull and "bleached" and the endosperm, as shown when the kernel is cut, changing from a clear vitreous to a soft white starchy appearance. There is thus a change in aspect of the grain from that of hard wheat to soft wheat and because this weathered wheat looked soft there was held, to some extent, the opinion that its quality had changed to that of soft wheat. It seems impossible, however, that the mere addition and removal of water should have so profound an effect as to change the inherent quality.

This problem was investigated by the Associate Committee on Grain Research, National Research Council of Canada (2), in the course of a study on the drying of wheat that had been exposed to varying degrees of weathering under known conditions. Some samples of damp wheat were stored in steel

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drums at outside temperature during the winter and then dried, milled and baked. The results obtained were not as decisive as might have been desired due to the fact that the prolonged exposure of the grain in the stook was favorable to sprouting and it was thought that this may have occurred to some extent. This of course would tend to obscure the effects of storage. There were observed a number of cases in which there was evidence of improvement and a number in which no apparent change took place. It could not be definitely determined whether or not the improvement were due to traces of sprouted grain and, therefore, the only conclusion reached was that "winter storage of damp sound wheat in steel drums of 40-80 gallons capacity, in no case resulted in significant deterioration of quality, and in some cases apparent improvement took place." The fact that those samples showing no change in quality had been stored at a moisture content lower than the others suggested that there might be a quite definite moisture limit below which no improvement could be expected. In order to investigate this question the work hereinafter described was undertaken.

Materials and Methods

Ten 20-lb. lots from a uniform sample of unweathered No. 2 Northern Marquis of 13.6% protein content were made up to moisture contents of 10, 11, 12, 13, 14, 15, 16, 18, 20 and 22% respectively. These moisture levels will hereinafter be designated by the numbers 1-10 respectively. These lots were then each subdivided into four 5-lb samples which were placed in cans just large enough to contain that quantity and the lids sealed thoroughly with paraffin. Two of the four series thus obtained were stored in a constant temperature room at 21°C. and the other two were placed outside on the north of the building. The experiment was started on December 1, so that those placed outside froze immediately and remained frozen during the term of storage.

After one month one of the series stored inside and one stored outside were opened, and those samples above 13% moisture were dried rapidly at room temperature by exposing them on mesh-bottomed trays over which a current of air was passed by means of an electric fan. They were then tempered and milled in the usual manner. The remaining two series were opened at the end of four months and treated similarly. The following designation of these series will facilitate reference to them:

Series I—No. 1-10 inclusive—Stored inside for one month
Series II—No. 1-10 inclusive—Stored outside for one month
Series III—No. 1-10 inclusive—Stored inside for four months
Series IV—No. 1-10 inclusive—Stored outside for four months

All samples except Nos. 9 and 10 of Series III appeared to be in good condition at the time the cans were opened. These two however were badly molded and quite out of condition. There was no evidence of heating in any of the samples stored at 21°C. The flours, milled to a 55% patent, were placed in cotton sacks immediately after milling and permitted to age for one

month, after which time they were transferred to half-gallon glass fruit jars and kept well sealed. Bakings were made in duplicate by the simple and bromate formulas using 50 gm. of flour and the procedure described by Larmour and MacLeod(1). In a second and third baking there was used, in addition to the simple and bromate, a blend formula involving the bromate formula applied to a mixture of 40% soft flour and 60% of the flour under examination. This will be referred to as the blend-bromate formula. These flours were baked after aging for one month, which means that Series III and IV (stored four months) were baked three months after Series I and II (stored one month). Then six months after the last baking, all forty samples were re-baked. At this time Series I and II had aged ten months and Series III and IV had aged seven months. Three months after this they were all baked again. As all three bakings showed the same general results, the average values were calculated and will be used in the following discussion.

Discussion of Results

Comparison of the four series may be made on the basis of the loaf volume and the baking score. For the sake of brevity the other bread scores have been omitted as they show nothing that is not to be found in these two.

TABLE I

COMPARISON OF LOAF VOLUME AND BAKING SCORE OF THE FOUR SERIES BAKED BY THE SIMPLE FORMULA

Sample No.	Moist. at which stored, %	Series I, inside, one month	Series II, outside, one month	Series III, inside, four months	Series IV, outside, four months
Loaf volume, cc.					
1	10	278	280	279	290
2	11	288	279	276	285
3	12	271	276	280	286
4	13	283	277	279	281
5	14	272	273	279	277
6	15	279	281	277	274
7	16	278	276	279	279
8	18	280	281	254	283
9	20	285	273	305	278
10	22	278	278	344	274
Baking score					
1	10	79	83	80	83
2	11	83	82	77	80
3	12	76	80	77	82
4	13	82	83	76	79
5	14	76	79	79	77
6	15	78	83	78	78
7	16	78	81	82	79
8	18	80	82	68	78
9	20	84	82	83	78
10	22	80	83	84	83

There was practically no change in texture, crumb color or general appearance of the loaves in the series, except in the case of Nos. 9 and 10, Series I, stored at 21°C. for four months with 20 and 22% moisture respectively. These samples were quite out of condition due to molding and gave bread of very poor color and texture. The loaf volumes and baking scores by the simple formula are given in Table I. These data show no evidence of any significant changes that might be attributed to differences in moisture content except in the case of No. 8, (moisture content, 18%), stored four months at 21°C. This sample was distinctly lower than the others in both loaf volume and baking score and it may be concluded that some damage to quality occurred with this treatment. Comparing the different series, there appears to be no significant differences between those stored at 21°C. and those stored at below 0°C. either for one month or for four months, nor does the difference in length of storage seem to have produced any definite changes.

The simple formula which involves use of flour, yeast, sugar, salt, and water only, is not very satisfactory for experimental flours, because it frequently fails to bring out the real strength and therefore often leads to false conclusions. For the purpose of evaluating wheat strength it is advisable to use some sort of improver or oxidizer in order to "loosen up" the gluten and permit the

TABLE II
COMPARISON OF LOAF VOLUME AND BAKING SCORE OF THE FOUR SERIES BAKED BY THE BROMATE FORMULA

Sample No.	Moist. at which stored, %	Series I inside, one month	Series II, outside, one month	Series III, inside, four months	Series IV, outside, four months
Loaf volume, cc.					
1	10	339	336	332	337
2	11	344	329	340	339
3	12	328	342	339	345
4	13	343	349	337	343
5	14	329	335	336	343
6	15	335	338	326	330
7	16	337	341	326	334
8	18	338	348	304	343
9	20	341	334	308	340
10	22	328	329	315	338
Baking score					
1	10	117	117	114	117
2	11	119	114	117	116
3	12	112	120	115	118
4	13	117	121	115	118
5	14	113	119	114	118
6	15	115	119	111	114
7	16	114	118	112	115
8	18	116	121	101	118
9	20	118	117	97	115
10	22	111	115	88	116

development of optimum hydration and elasticity. In this study, however, the simple formula was used in order to see if the treatments given the samples would show evidences of modification of the gluten. Saunders, Nichols, and Cowan (3) reported that successive wettings and dryings of wheat caused a change in the wheat which resulted in improved baking properties when a formula similar to the simple formula was used. It was thought that keeping the wheat damp for as long a period as four months might produce somewhat similar results. The data however fail to show any tendency toward improvement.

The results obtained by use of the bromate formula are given in Table II. The values were remarkably constant except in the cases of Nos. 8, 9, and 10, Series III, samples with moisture contents of 18, 20 and 22% respectively and stored at 21°C. for four months. In these samples there was undoubtedly lowering of both loaf volume and baking score. The 18% sample on inspection showed no evidence of damage but it was unmistakably lower in baking quality than the preceding members of Series III (stored inside for four months). It should be noted here that the following two members of this series, which did not show evidence of damage when baked by the simple formula, were by the bromate formula distinctly lower in quality than the

TABLE III

COMPARISON OF LOAF VOLUME AND BAKING SCORE OF THE FOUR SERIES BAKED BY THE BLEND-BROMATE FORMULA

Sample No.	Moist. at which stored, %	Series I, inside, one month	Series II, outside, one month	Series III, inside, four months	Series IV, outside, four months
Loaf volume, cc.					
1	10	299	300	296	306
2	11	310	298	299	300
3	12	302	305	298	290
4	13	314	303	295	302
5	14	298	303	297	301
6	15	305	306	296	305
7	16	301	296	285	299
8	18	309	307	277	300
9	20	305	296	268	301
10	22	302	300	306	293
Baking score					
1	10	96	97	94	98
2	11	100	92	92	95
3	12	94	96	93	92
4	13	100	97	92	95
5	14	93	98	93	95
6	15	97	99	94	97
7	16	94	93	87	95
8	18	99	97	82	95
9	20	98	92	73	95
10	22	95	95	76	91

upper members of the series. All the other samples exhibit variations no greater than might be expected in replicate bakings of one sample of flour.

A blend of soft flour with the flour under investigation baked by the bromate formula frequently brings to light differences in quality not apparent from the data obtained by either the simple or bromate formula. In Table III however the only additional fact revealed by this blend-bromate formula was that sample No. 7, as well as Nos. 8, 9, and 10, Series III, was low in quality. No. 10, Series IV, which was stored at below 0°C. with 22% moisture was slightly lower in loaf volume and baking score than those immediately preceding it in Series IV, but this cannot be considered significant because No. 3 stored with only 12% moisture showed nearly identical values.

Summing up the results of the baking tests by all three formulas, only the samples with 16, 18, 20 and 22% moisture, stored at 21°C. for four months, showed evidence of deterioration. All the others exhibited differences no greater than would be expected from replicate bakings of one flour. This indicates that if wheat at moisture content up to 22% can be stored at or below 21°C. in quantities small enough to avoid heating no measurable change in quality will occur in one month. If the wheat can be kept at below 0°C. (32°F). no change occurs during a period of four months. When the moisture exceeds 15%, samples stored at 21°C. (70°F.) for four months undergo deterioration in quality, the extent of which increases with the moisture.

References

1. LARMOUR, R. K. and MACLEOD, A. G. Sci. Agr. 9: 477-490. 1929.
2. NATIONAL RESEARCH COUNCIL OF CANADA. Report No. 24. 1929.
3. SAUNDERS, C. E., NICHOLS, R. W. and COWAN, P. R. Can. Exptl. Farm Bull. 97. 1921.

THE TRIAENOPHORUS PARASITE IN THE FLESH OF THE TULLIBEE (LEUCICHTHYS)[1]

By Daniel Nicholson[2]

Abstract

In the flesh of many tullibee and some whitefish there are yellow cysts which contain a thread-like worm 5 to 25 cm. (2 to 10 in.) in length. As these fish are favorite foods, a study of the characteristics and pathogenicity of any larvae found in their flesh is important. This paper describes the appearance of the parasite and relates it to its adult form in the intestine of the jackfish (*Esox lucius*) which preys on young tullibee and white fish. Feeding the parasite to dogs did not result in the development of adult tapeworms and from this it is inferred that triaenophorous parasite is non-pathogenic to man.

Incidence of Triaenophorus Infestation

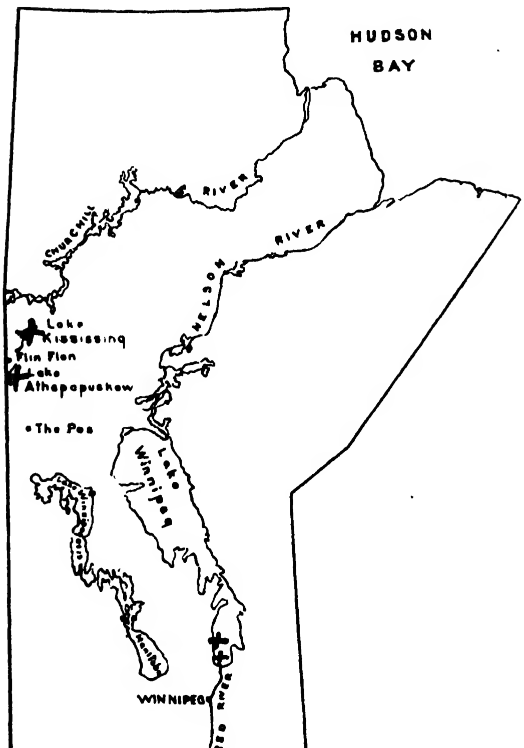

FIG. 1. *Map of Manitoba Lakes. The tullibee examined were caught in the localities marked with a cross.*

The first lot of 48 tullibee examined were caught in Lake Winnipeg during September 1929. They were identified by Dr. A. Bajkov as *Leucichthys nigripinnis* and *Leucichthys tullibee.* Forty-one were infested, each containing from 2 to 18 parasites. In another lot of 60 tullibee caught during May 1920, 46 were infected, each containing from one to ten parasites.

Out of 23 whitefish (*Coregonus clupeaformis*) caught during January 1931 in Kississing Lake, which is north of the Pas, 18 were infected with triaenophorus parasites. Of twenty whitefish caught in Lake Athapapuskow about the same time only one was infected with triaenophorus.

There are five varieties of tullibee in Lake Winnipeg and from extensive investigation of the incidence of infestation and feeding habits of each of these

[1] *Manuscript received July 14, 1931.*
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species, Dr. Bajkov finds that the black backed tullibee (*L. tullibee*), of which 60% are infected with triaenophorus parasites, feed mainly on the bottom fauna of the lakes, together with some of the surface plankton. The black fin (*L. nigripinnus*), the small bloater (*L. hoyi*), and the black back nipigon tullibee (*L. nipigon*), are almost 100% infected and they feed mainly on plankton which is near the surface. The long jaw (light back) tullibee, (*L. zenithicus*) feeds on bottom fauna and is very lightly infected. About 25% contain one, and rarely more than two, parasites. The whitefish, which is closely related to the long jaw (light back) tullibee and also a bottom feeder, is slightly infected. About 10% of them contain a few parasites. The breeding of the light back tullibee in hatcheries would tend to lessen infestation.

Morphology of the Triaenophorus Parasite

To examine the fish, it is placed on its side, the skin slit longitudinally near the dorsal fin and again on the lateral side of the belly. The skin can then be peeled off from head to tail. With a long, sharp knife thin slices are pared off.

In a few, the cysts containing the triaenophorus are thin-walled and transparent. These always contain plump, active parasites. In most fish the cyst wall is yellowish and moderately tough; this type of cyst frequently contained a yellowish milky fluid surrounding the parasite.

Microscopic examination of this fluid showed it to be amorphous material as shown in Fig. 5. No tissue cells or bacteria were present. The triaenophorus in this type of cyst was slender and much coiled and many of them had no movement. This would suggest that the cyst wall developed from irritation by the parasite. The triaenophorus parasite ranges in length from 5 to 25 cm. (2 to 10 in.) and in diameter from 1 to 3 mm. The body is round or ovoid and the segments are indistinctly marked in contrast to the adult tapeworm form. Most of the parasites were quite active and crawled entirely out of the cyst when it was opened up. The head showed a very definite structure with two trident shaped hooklets on each side (Fig. 6.) The similarity of this to the head of the tapeworm found in the intestine of the pike is very striking (*cf.* Fig. 7).

Feeding Experiments to Determine the Effect of the Triaenophorus on Mammals

As it is very important to determine whether these parasites are detrimental to mammals, the author prepared three dogs by examining the feces for ova and parasites before and after dosing with felix mas. This being negative, the dogs were then fed slices of fish containing triaenophorus parasites. One dog was fed 12, another 18 and a third, 24 parasites. After this they were fed wholly on biscuits for a month and they thrived well. One month after the feeding, chloroform was administered and a necropsy performed. There were no tape or round worm parasites in the intestinal tract. The other

internal organs and the skeletal muscles were carefully examined by making thin slices and inspecting under a hand lens. No lesions or parasites could be seen.

In May 1930, three dogs were again subjected to feeding experiments using technique similar to that in the previous test, except that the dogs were fed more parasites. Twenty-four were fed to one dog, 36 to the second and 48 to the third. Post-mortem examination one month later revealed no intestinal parasites or infestation of the internal organs or skeletal muscles. The fact that the larval form of the triaenophorus parasite did not cause infestation in dogs would indicate that other mammals including man were not capable of being infested.

Other Parts of the Triaenophorus Life Cycle

(a) *As an Adult Tapeworm in the Intestine of the Pike*

During 1927 and 1928 while examining pike (*Esox lucius*) for parasites (*Diphyllobothrium latum*), the author was surprised to find that the intestine of the pike contained many small tapeworms having two broad, trident-shaped hooklets on each side of the head. The body possessed well-marked segments and the mature end segments contained a uterus from which tiny eggs could be extruded on pressing the segment between two glass slides. This parasite was identified by Dr. Bajkov as a triaenophorus and a photograph of the head was included in the article then published on fish tapeworm. It is reproduced here in Fig. 7, to show its similarity to the parasite found in the flesh of the tullibee.

The jackfish or pike (*Esox*) is sometimes called the fresh-water shark. It preys on other fish, frequently on tullibee and whitefish. If these are infested with triaenophorus larvae, the identical appearance of the heads and hooklets would suggest that the small adult tapeworm in the intestine of the pike grows from the larval form in the flesh of the devoured tullibee. The segments in the adult worm are more distinct and flatter than in the larval forms and the ripe terminal ones extrude eggs.

(b) *Food of Tullibee and Whitefish as a Link in the Life Cycle*

The steps in the life cycle are unknown from the extrusion of the triaenophorus egg with the feces of the pike to the infestation of the tullibee by the larval plerocercoid. Food is the most likely source of infestation and the tullibee and whitefish do not eat other fish but live entirely on the plankton and bottom fauna, the minute forms of animal life that abound in lake waters. If the plankton become infested by ingesting triaenophorus eggs or their embryos, they would be the most likely link in the infestation of the tullibee. From his extensive investigations of the food of various species of fish Dr. Bajkov is of the opinion that some of the small crustaceans probably, *Limnocalanus macrurus*, *Epischura lacustris*, *Diaptomus scilis* or *Mysis relicta* may be the intermediate hosts between the triaenophorus egg extruded in the feces of the pike and the larval form in the flesh of the tullibee.

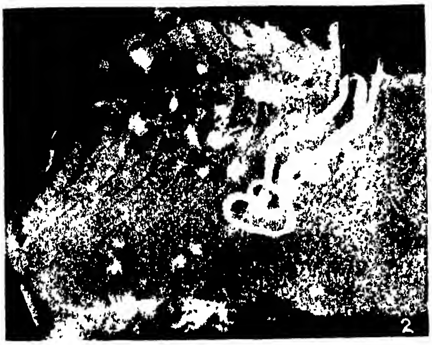

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FIG. 2. *Slice of tullibee showing a large active triaenophorus parasite which has crawled out of the opened cyst just below the coils. The white spots on the fish are drops of bichloride used to arrest the movement of the parasite for purposes of photography. Natural size ×0.7.*

FIG. 3. *Thick walled cysts containing triaenophorus (above) and long slender triaenophorus (below) contained in such cysts. Natural size ×0.7.*

FIG. 4. *A section through a cyst showing the surrounding muscle, a thin cyst wall and some sections of the parasite. The fluid contents of the cyst have drained away. ×6.*

FIG. 5. *Section through the wall of a cyst showing the character of the contents above and the fish muscle below. There are no bacteria or pus cells present in this exudate. ×130.*

FIG. 6. *Head of a large triaenophorus larval parasite from tullibee. The head is slightly pressed between two glass slides and turned up to show the hooklets on both sides. ×9.*

FIG. 7. *Head of adult triaenophorus tapeworm from intestine of the pike. It shows the resemblance to the head of the larval form in Fig. 6. The hooklets on the posterior side show through just above and to the right of the hooklets on the proximal side. ×19. Reproduced from paper cited (2).*

LIFE CYCLE OF TRIAENOPHORUS PARASITE

$$\begin{array}{ccc} \text{Larvae (plerocercoid) in flesh of tullibee and whitefish} & \longrightarrow & \text{Adult tapeworm in intestine of pike} \\ \uparrow & & \downarrow \\ \text{Infected plankton. Crustacea containing procercoids in shallow water ?} & \longleftarrow & \text{Eggs in feces of pike} \end{array}$$

Conclusions

1. The adult tapeworm in the intestine of the pike resembles the larval plerocercoid in the flesh of the tullibee and likely develops through the pike eating infested tullibee.

2. The triaenophorus parasite which infests the flesh of tullibee and whitefish is non-pathogenic to dogs and likely also to all mammals. Its appearance is objectionable.

3. The practical difficulties of clearing the lakes of jackfish (*E. lucius*) should be carefully investigated. This fish is the commonest host of Triaenophorus and Diphyllobothrium parasites. It eats other valuable fish and itself has little market value. A bounty for *Esox* caught up creek at spawning time or an industry that could utilize them for fish meal or fertilizer might be considered.

Acknowledgments

The author is much indebted to Dr. Alexander Bajkov for identifying the varieties of fish and in giving him the benefit of his wide experience in the incidence of infestation and feeding habits of tullibee and whitefish. Dr. Moorhouse and Dr. Ormerod, Department of Physiology, University of Manitoba, very kindly gave their facilities for the dog feeding experiments.

References

1. BAJKOV, A. Biological conditions of Manitoban lakes. Contr. Can. Biol. Fisheries, v.5 no. 12. 1930.
2. NICHOLSON, D. Fish tapeworm. Intestinal infection in man: the infestation of fish in Manitoba lakes. Can. Med. Assocn. J. 19: 25-33. 1928.

DIPHYLLOBOTHRIUM INFECTION IN *ESOX LUCIUS*[1]

By Daniel Nicholson[2]

Abstract

This study showed that most of the summer caught jackfish or pike (*Esox lucius*) contained many larvae of *D. latum* (plerocercoids), in contrast to the winter caught fish which showed a scanty infestation. The larvae in the winter caught fish were small and frequently motionless, while those in the summer caught fish were large and quite active.

When dogs were fed *motile* larvae from winter caught fish, very few adult tapeworms developed. Sixty per cent of the larvae contained in the summer caught fish produced adult tapeworms. This suggests that *D. latum* infection of the *Esox* fish is probably an annual event occurring in the early summer months.

It is the small to medium sized *Esox* that contain numerous *D. latum* parasites. The very large fish contained so few parasites as to suggest that after a period of development the *D. latum* larvae (plerocercoid) in the flesh of the fish die off and disintegrate leaving no trace of their presence, and as the fish become large the procercoid form of the parasite has difficulty in penetrating the intestinal wall of the fish.

Larvae in Summer Caught Esox

The larvae in the flesh of summer caught *Esox* are very numerous. Most of them measure ½ to ¾ in. in length and have some of the characteristics of mature tapeworms, such as the slit head and rudimentary segments. They are quite active and when placed on the wetted surface of the hand, crawl about vigorously. Even after ordinary cold storage freezing, the larvae when warmed exhibit this activity.

Larvae in Winter Caught Esox

There are very few larvae (plerocercoids) in the flesh of *Esox* caught during March, April and May, as shown in Table I. Those found are very small and many are degenerated so that when moved with a probe they break up. Of those that remained intact on probing only 9% showed movement, which was quite sluggish in character. In those that are motile the segments are quite rudimentary and the slit in the head is only moderately developed.

The findings in Table I would suggest that infection of *Esox* with *D. latum* parasites takes place in the summer and that the parasites die off after five to six months.

Infestation in Large Fish

Fifty-two per cent of the *Esox* 10 to 18 in. in length harbored parasites which were frequently very numerous. As many as 63 were counted in one fish. It was very striking that only a few of the *Esox* over 24 in. long harbored parasites, nor was there the slightest macroscopic trace of past infestations. Classified from the standpoint of size, only 3% of the fish over 24 in. long harbored parasites and then only one or two small parasites were found.

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Contribution from the Department of Pathology, University of Manitoba, Winnipeg, Manitoba, with financial assistance from the National Research Council of Canada.

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TABLE I

DEGREE OF INFESTATION OF *Esox* CAUGHT IN LAKE WINNIPEG, AUGUST 1928 TO JUNE 1931

Date	Number examined	No. containing larvae	Degree of infestation	State of larvae	Date	Number examined	No. containing larvae	Degree of infestation	State of larvae
1928					1930				
Aug.	48	43	C	X	Jan.	60	21	B	YZ
Sept.	36	35	C	X	Feb.	84	12	AB	YZ
Oct.	24	21	C	X	Mar.	120	3	A	Z
Nov.	24	19	B	XY	April	96	2	A	Z
Dec.	60	34	B	XZ	May	96	6	B	Y
					July	24	19	C	XY
1929					Aug.	30	28	C	X
Jan.	72	21	AB	YZ	Oct.	12	12	C	X
Feb.	80	17	A	YZ	Nov.	18	15	C	XY
Mar.	96	0	O		Dec.	24	14	BC	YZ
Apr.	120	6	A	YZ					
Aug.	30	28	C	X	1931				
Sept.	18	18	C	X	Jan.	36	13	BA	YZ
Oct.	18	16	C	X	Feb.	60	10	BA	YZ
Nov.	36	31	C	XY	Mar.	72	4	A	Z
Dec.	48	37	CB	Y	Apr.	60	3	A	Z
					May	44	5	AB	Y
					June	32	9	B	Y

NOTE:—*Degree of infestation: O = No larvae; A = 1 to 3 larvae; B = 3 to 12 larvae; C = over 12 larvae. State of larvae: X = Very active, large; Y = Sluggishly active, small; Z = No motion, some degenerated.*

It is hardly reasonable to suppose that these large fish have been free from parasites in all the previous summers of their existence. Moreover the intestinal wall of large *Esox* must offer more resistance to the procercoid of the *D. latum* larvae than the intestinal wall of the smaller fish. They are both caught in the same waters and their stomach contents are similar. All this is considerable evidence that the infestation of *Esox* by *D. latum* parasites is not permanent.

Feeding Experiments to Determine Viability of Parasites

To test the ability of the parasites to develop into adult tapeworms three dogs were fed with *motile* larvae of *D. latum* from winter caught *Esox*. One was given 5, another 10 and the third 15 larvae from fish caught during January and February 1929. Before infesting the dogs they were carefully examined and dosed with felix mas to make sure that they harbored no parasites. After eating the larvae the dogs were fed wholly on biscuits and water for a period of a month. A post-mortem examination was then made. The dog that had eaten five motile larvae of *D. latum* had one adult tapeworm (*D. latum*). The second dog that had eaten 10 larvae showed 5 adult tapeworms and the one that had eaten 15 showed 4. Thus only 27% of the motile larvae from winter caught fish when fed to dogs developed into adult tapeworms.

The experiment was repeated in August 1930, feeding the larvae from summer caught fish to dogs according to the routine already outlined. One

dog ate five large vigorous larvae from summer caught fish and developed four tapeworms (*D. latum*). Another ate 10 summer larvae and developed 6 tapeworms. A third ate 15 larvae and developed 8 tapeworms. These dogs lost weight rapidly toward the end of the experiment. Thus 60% of the larvae from summer caught fish developed into tapeworms.

The infestation from winter caught fish is really much lower than the above experiment would suggest when one remembers that very few winter caught fish are infested, and of the few larvae found only an occasional one shows any motility, and of the ones showing motility only 27% developed into adult tapeworms. This is quite in agreement with the views of Indians and fishermen around Lake Winnipeg who say that winter caught jackfish (*Esox*) are not harmful to their dogs but that if the latter are fed on jackfish caught in summer, they will develop worms. During the latter part of the summer it is not an uncommon sight around Lake Winnipeg to see dogs that have parasites protruding from the rectum, and this would suggest that dogs are the most important mammalian link in the life cycle of the *D. latum* parasite. Many of the eggs from the parasites in dogs are fertile and when incubated develop into swimming embryos.

Temperatures Required to Kill Parasites

Batches of 12 summer caught fish were rolled in a paper and placed in cold storage at varying temperatures for 24 hr. When the lowest point registered was $-11°$ C. ($+12.2°$ F.), by a minimum recording thermometer placed among the fish, all parasites were killed. The parasites were not killed in fish that were stored in cold storage for 24 hr. where the minimum temperature recorded in the same manner was $-3°$ C. ($+26.6°$ F.). During these experiments no record was kept of the rapidity with which the minimum temperature was reached. In all experiments the minimum temperature recorded was present when the fish were removed. If many fish are packed closely, the low temperature takes longer to penetrate than when fish are in a small package. Rapid changes in temperatures are more fatal to life than gradual changes and the duration of the low temperature is important. Further experiments should be conducted to find out the time necessary to lower the temperature in the centre of a large pile of boxes of fish before this measure could be adapted for commercial purposes. While the cooking of fish kills all parasites, should the *Esox* or the *Leucioperca vitreum* (pickerel) be objected to on the ground that they harbor dangerous parasites, this objection could be overcome by chilling to $-11°$ C. for 24 hr.

Discussion

The pike which were the subject of this study were caught in Lake Winnipeg, which is about 240 miles long, extending from a southern latitude of 50.5° in a northern and slightly western direction to a latitude of 53.7°. The north end of the lake has a longer and colder winter season and its surrounding country is sparsely inhabited. All except the catch of August, September and October 1928 were taken from the southern arm of the lake which forms a large bay almost 100 miles long. The country surrounding this part of the lake is well

settled and the sewage from the City of Winnipeg, some 40 miles distant, drains by way of the Red River into the south end of the lake.

It was not possible to obtain fish from a single area, although it would have been ideal for this study. The fish examined were those supplied for sale in the open market and the author was informed by Mr. Roundtree of the Winnipeg Fish Company that many of the summer caught fish are obtained near the shore while the winter caught fish are usually taken through the ice farther off shore. Most of the summer caught fish were taken from the vicinity of Matlock. Dr. Alexander Bajkov has informed the author that there is a lagoon near Gimli in which *Esox* are more heavily infested even in the winter than are the fish caught in the other parts of the lakeshore. He attributes this to the stillness of the water, the many dogs around this area which infest the water with ova of *D. latum*, and the fact that the water is shallow and warmer than in the main lake.

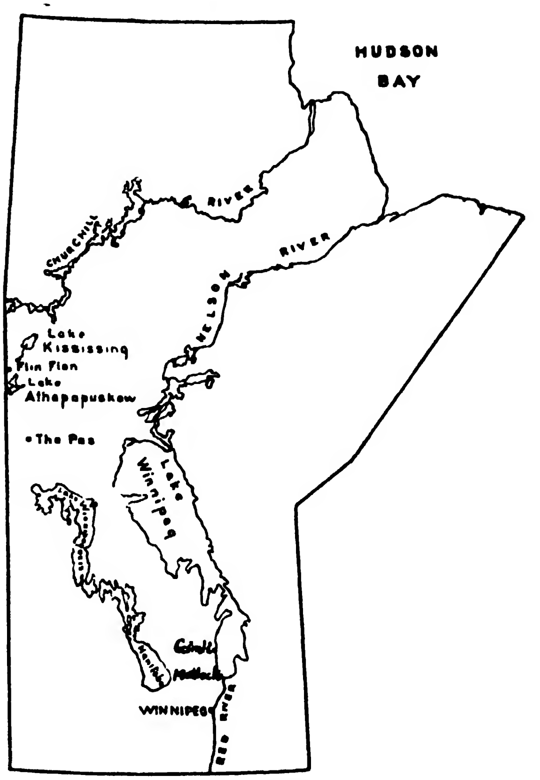

FIG. 1. *Map of Lake Winnipeg.*

During the winter, low temperatures kill ova deposited on excreta on the ice and the water is too cold for any of the embryos to hatch from eggs that might be brought in by running streams or sewage.

In April and May the water warms sufficiently to allow ova of *D. latum* to develop into embryos. These embryos are eaten by diatoms which in turn are ingested by the fish.

Conclusions

1. The numerous large active plerocercoid larvae of *D. latum* found in *Esox* during the summer, when contrasted with the infrequent, inactive and sometimes degenerated larvae found during the winter and spring, suggest that the source of infestation is more prevalent during the summer months.

2. The parasite after living in the muscle for several months, shrivels up and degenerates. Large fish rarely harbor parasites.

3. The fishing of *Esox* which are to be marketed should be done during the late winter and spring months and summer fishing prohibited.

4. Freezing to $-11°$ C. will kill the larvae of *D. latum* in the *Esox*.

5. As dogs are an important link in the infestation, those harboring parasites should be dosed with malefern and advice given to their owners regarding the prevention of further infestation. Dogs should not be fed on raw summer caught fish.

6. As the *Esox* has little or no market value, means of getting rid of it in lakes should be considered. A bounty might be offered for fish caught up creeks at spawning time or it would be well to consider an industry that could make use of them for fishmeal or fertilizer. This would also break the life cycle of the triaenophorus parasite which inhabits the intestines of the *Esox* as a tapeworm, and spends its larval stage in the flesh of tullibee, whitefish and other species that have good market value.

Acknowledgments

The author is indebted to the late Mr. Arthur Rigby, food inspector, Winnipeg Health Department and to Mr. G. N. Rowntree, Manager of the Winnipeg Fish Co., for supplying fish together with information regarding their origin. Dr. V. H. K. Moorhouse and Dr. M. J. Ormerod, Department of Physiology, University of Manitoba, kindly gave facilities for the dog feeding experiments.

References

1. BAJKOV, A. Biological conditions of Manitoban lakes. Contr. Can. Biol. Fisheries, v. 5, no. 12. 1930.
2. NICHOLSON, D. Fish tapeworm. Intestinal infection in man: the infestation of fish in Manitoba Lakes. Can. Med. Assocn. J. 19: 25-33. 1928.
3. NICHOLSON, D. Variations in the fish larvae of *Diphyllobothrium latum*. Can. Public Health Journal. 20: 193-195. 1929.

INHERITANCE OF RESISTANCE TO FOWL PARALYSIS (*NEUROLYMPHOMATOSIS GALLINARUM*)

I. DIFFERENCES IN SUSCEPTIBILITY[1]

By V. S. Asmundson[2] and Jacob Biely[3]

Abstract

Data on the incidence of paralysis and lymphomatous tumors in a flock of 542 pullets of six different breeds indicate the presence of both in 14 out of 52 paralyzed pullets, while an additional 22 had tumors but were not paralyzed. The association of paralysis and tumors may have been due to chance. The evidence presented, while not conclusive, points to the inheritance of resistance to paralysis. This is indicated particularly by (1) differences in the proportion of paralyzed pullets in different breeds, and (2) absence of paralysis among the progeny of certain males and in certain large families. The data obtained point to a simple mode of inheritance.

Among the important problems facing the poultry farmer is the control of disease. Since it is now generally accepted that resistance to disease depends, in some cases at least, upon hereditary factors, it follows that one of the methods of controlling losses from disease is to breed resistant strains. Considerable progress along this line has been made with plants. Much research has also been conducted on the inheritance of disease resistance in animals and the breeding of disease resistant strains of domestic animals has been started.

The three projects with domestic fowls that have, so far, been reported on are: (*a*) Inheritance of resistance to diphtheria (5); (*b*) Inheritance of resistance to fowl typhoid (6, 7); and (*c*) Inheritance of resistance to pullorum disease (1). Frateur (5) found evidence that resistance to diphtheria was determined by a single dominant factor. Lambert and Knox (6, 7) hatched chicks from unexposed parents and from parents that had survived an acute infection of fowl typhoid. The mortality among chicks from unexposed parents was 41% as compared with a mortality of 90% in the case of chicks from unexposed parents. They consider that resistance is inherited on the basis of multiple factors. The data of Card and Roberts (1) show fairly clearly that resistance and susceptibility to pullorum disease are, at least in part, due to the existence of hereditary factors. In addition to these reports Mathews (8) found some evidence "that heredity is associated with the occurrence of leukochloroma." Mathews and Walkey (9) have presented evidence in support of the theory "that the lymphoid neoplasms are inherited as a mendelian recessive characteristic."

As yet, no attempt has been made to study the inheritance of resistance to fowl paralysis, although it is generally recognized that the widespread and frequent outbreaks of paralysis cause tremendous losses to the poultry industry.

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Contribution from the Departments of Poultry Husbandry and Bacteriology, University of British Columbia, Vancouver, Canada, with financial assistance from the National Research Council of Canada.

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Pappenheimer, Dunn and Cone (12) report that mortality may range from a few per cent up to 60% and over. Furthermore, the disease recurs year after year.

Pappenheimer *et al.* (12) noted what they called a "very significant indication of a breed or varietal difference in susceptibility to paralysis." They state that 14 out of 27 Silver Spangled Hamburg chicks died from paralysis, while other chicks kept under the same conditions and at the same time, did not develop paralysis.

Doyle (2, 3) has obtained evidence which, he considers, shows that fowl paralysis is hereditary. He found that chicks hatched out of eggs from flocks in which paralysis was present have a tendency to develop paralysis.

Thomas (13) states that fowl paralysis is transmitted with great difficulty and that a hypothesis of individual susceptibility, or alternately, general natural resistance, has been advanced to explain this.

Experimental

The data reported in this paper are from a flock of 542 pullets comprising six breeds. These had been hatched and reared together. A few cases of paralysis occurred before the pullets were banded and removed to the laying houses, but all those banded were apparently in good health. The losses in this case thus occurred somewhat later than usual. The clinical and pathological symptoms, which will be described in detail in a subsequent paper, have been found to correspond with those described by Pappenheimer *et al.* (12), Doyle (2, 3), Dobberstein and Haupt (4), Thomas (13), McGaughey and Downie (10).

Pappenheimer *et al.* presented evidence in favor of the view that the association of fowl paralysis with visceral lymphomata, originating in the ovary, is not accidental, and that the lymphomata are a manifestation of the disease. The data for this flock that have a bearing on this problem are shown in Table I.

TABLE I
DISTRIBUTION OF PARALYSIS AND LYMPHOMATOUS TUMORS BY BREEDS

Breed	No. of pullets in families free from tumor and paralysis	No. of pullets in families in which paralysis or tumors were present			
		Normal	Paralysis only	Tumors only	Paralysis and tumors
S.C.R.I. Reds	49	51	13	2	4
B.P. Rocks	64	25	4	3	1
White Wyandottes	33	21	7	2	
White Wyandottes*		50	2	6	3
Australorps	34	17	2	1	
Light Sussex	21	3			1
S.C.W. Leghorns	39	35	10	8	5
Total	240	202	38	22	14

* *Unpedigreed birds from flock matings.*

It will be seen from this table that, of the 516 birds for which some information is available (on 25 birds no post-mortem examination was made), 52 were paralyzed and 36 had tumors. Fourteen of these had tumors and were paralyzed. On the basis of the total population of 516 pullets, the number of cases where both occur together is 3.8 times the number expected as a result of chance. Presumably the 240 pullets in families free from paralysis and lymphomatosis were resistant and may, on that assumption, be not considered. When the calculation is based on the remaining 276 pullets belonging to families or groups in which some paralysis or tumors occurred, the ratio becomes 2:1. The simultaneous occurrence of tumors and paralysis in these 14 birds may, therefore, be due to chance. Accordingly, only the cases of actual paralysis are included in Table II, but it should be stated that the interpretation of the data in this table would not be materially changed by grouping the birds having lymphomata with the paralyzed birds.

TABLE II

SUMMARY OF ALL FAMILIES OF PULLETS HATCHED IN 1930 SHOWING INCIDENCE OF PARALYSIS

Sire No.	All of pullet progeny normal		Part of pullet progeny paralyzed			Died, no post-mortem
	No. of dams (families)	No. of pullets kept	No. of dams (families)	No. of pullets kept		
				Normal	Paralysis	
1[1]	2	6	3	10	7	3
2[1]	1	4	3	19	6	1
3[1]	4	18	2	16	2	1
4[1]	3	15				
5[1]	2	10	2	4	2	
6[2]	3	3	1	1	1	
7[2]	7	23				
8[2]	6	26	1	3	1	1
9[2]	2	8	2	10	3	2
10[2]	5	13				
11[2]	2	5				
12[3]	2	9	6	23	7	5
13[3]	7	24				5
14[4]			?	56*	5*	1
15[5]	4	23	1	12	1	
16[5]			1		1	
17[5]	4	17				3
18[6]	4	19				
19[6]	1	2	1	3	1	
20[7]	4	10	4	13	5	
21[7]	2	4				1
22[7]	2	9	2	3	2	
23[7]	5	20	2	2	2	2
24[7]	4	12				
25[7]	1	1	4	4	4	
26[7]			2	4	2	
Totals		281		127	47	25

1—S.C.R.I. Reds; 2—B.P. Rocks; 3—White Wyandottes; 4—White Wyandottes from a flock mating; 5—Australorps; 6—Light Sussex; 7—S.C.W. Leghorns.

**Not included in totals.*

Incidence of Paralysis

The losses sustained from paralysis among the progeny of different males, grouped according to breed, are shown in Table II. This table is based on cases occurring after the end of September when the birds were about six months old. Since on 25 birds no post-mortem examination was made and others were sent to market during the latter part of the year, this table may not show the total possible cases of paralysis. Accurate information is, however, available for the period of heaviest losses from paralysis, hence there is every reason to believe that it shows the relative incidence of paralysis fairly accurately. On account of the small number of pullets in any one family it was not considered advisable to present the details for each family.

There was a difference in the proportion of paralyzed pullets of the various breeds. The percentages in each breed were as follows:

Australorps, 3.7%; Light Sussex, 4.0%; B.P. Rocks, 5.2%; W. Wyandottes, 9.7%; S.C.R.I. Reds, 14.3%; S.C.W. Leghorns, 15.5%.

Since the number of birds, in the case of some of these breeds, was relatively small, it cannot be assumed that real breed differences are involved, but the variation in the percentage of birds affected may indicate differences in the strains and groups here used.

There appears to be a difference in susceptibility to paralysis among the progeny of different families (Table II). Unless there is a difference in susceptibility, it is difficult to account for the absence of paralyzed pullets among the daughters of some of the males. This might be due to chance in the case of males Nos. 11 and 21, since there were only five and four daughters respectively, but it is not likely that chance alone is responsible for the absence of paralyzed pullets among the progeny of males Nos. 4, 7, 10, 13, 17, 18 and 24. The proportion of paralyzed to normal daughters from other males was 1:6. (47 paralyzed to 276 normal). The proportion in families where some paralysis occurred was higher or approximately 1 paralyzed to 3 normal (47 paralyzed to 127 normal, Table II). Assuming on this basis and for the sake of simplicity that one-fourth of the progeny of males Nos. 4, 7, 10, 13, 17, 18 and 24 would have become paralyzed if chance alone governed the results, there should have been from three to six paralyzed daughters from each of these males. The deviation from 3:1 ratio, the probable error, Dev./P.E. and odds against these deviations being due to chance for the progeny of these seven males are as shown in Table III.

TABLE III

Male No.	No. of progeny	Dev.	P. E.	Dev./P.E.	Odds
4	15	3.75	1.13	3.3	37.4:1
7	23	5.5	1.37	4.0	142.3:1
10	13	3.25	1.05	3.1	26.4:1
13	24	6.00	1.43	4.2	215.8:1
17	17	4.25	1.24	3.4	44.8:1
18	19	4.75	1.27	3.7	78.5:1
24	12	3.0	1.01	3.0	22.2:1

The odds against these deviations from an expected 3:1 ratio being due to chance alone vary from 22:1 to 216:1. Some allowance should perhaps be made for the fact that the proportion of paralyzed daughters among the progeny of other males was less than one-fourth. Even if this is done and a further allowance made for deaths from undetermined causes among the progeny of males Nos. 13 and 17 (see Table II), the evidence here presented is such as to indicate that the absence of paralysis in the progeny of these seven males is not due to chance alone. These results, therefore, point to inherent differences as an important factor in resistance to paralysis. In further support of this conclusion it is of interest to note that there were several families comprising 7 to 11 pullets, none of which became paralyzed, although pullets from the same sire but a different dam were affected.

Mode of Inheritance

Considering next the possible basis of inheritance of resistance to paralysis, it will be noted (Table II) that slightly over one-fourth of the pullets in these families became paralyzed. Assuming a single factor difference, this proportion of affected pullets would indicate that the parents were heterozygous for the gene for susceptilibity to paralysis. Since this was apparently the first time that the flock had been exposed to paralysis, some of the parents would be expected to be susceptible and, as a result, half or all of their progeny would be susceptible, depending upon the constitution of the other parent. An examination of the individual families shows that in about 12 families the number of paralyzed and non-paralyzed pullets is equal, thus pointing to a 1:1 ratio. All of these are small families of not more than five pullets. There were, in addition, three families of one pullet each which were paralyzed, but the numbers here are too small to furnish critical evidence. The data indicate that resistant birds differ from susceptible birds by a single dominant gene, but since other factors besides heredity may influence the incidence of paralysis, no conclusion can, in the present state of knowledge, be drawn with respect to this point. If the mode of inheritance of resistance to paralysis is simple it should be a relatively easy matter to breed strains of domestic fowl that are highly resistant to paralysis. The greatest obstacle at present is the difficulty of reproducing the disease under laboratory conditions. Further work on these phases of the problem is now in progress.

References

1. Card, L. E. and Roberts, E. Proc. Fourth World's Poultry Congress (Lond.). Sec. C. 526-533. 1930.
2. Doyle, L. P. J. Am. Vet. Med. Assocn. 68, n.s. 21: 622-630. 1926.
3. Doyle, L. P. J. Am. Vet. Med. Assocn. 72, n.s. 25: 585-587. 1928.
4. Dobberstein, J. and Haupt, H. Z. Infectionskrankh. Haustiere, 31: 58-80. 1927.
5. Frateur, J. L. Proc. Second World's Poultry Congress (Barcelona). Sec. 1. 68-71. 1924.
6. Lambert, W. V. and Knox, C. W. Iowa State College, J. Sci. 2: 179-187. 1928.
7. Lambert, W. V. Sci. Monthly, 28: 118-121. 1929.
8. Mathews, F. P. Arch. path. 7: 442-457. 1929.
9. Mathews, F. P. and Walkey, F. L. J. Cancer Research, 13: 383-400. 1929.

10. McGaughey, C. A. and Downie, A. W. J. Compt. Path. Therap. 43: 63-76. 1930.
11. Pappenheimer, A. M., Dunn, L. C. and Cone, V. C. J. Exptl. Med. 49: 63-86. 1929.
12. Pappenheimer, A. M., Dunn, L. C. and Cone, V. C. Storrs Agr. Exptl. Sta. Bull. 143: 187-290. 1926.
13. Thomas, A. D. J. S. African Vet. Med. Assocn. 67: 178-183. 1928.

STUDIES ON B.C.G. VACCINE

II. NON-VIRULENCE AND RESISTANCE IN NEW-BORN CALVES[1]

By A. C. Rankin[2], J. J. Ower[3], R. M. Shaw[4], P. R. Talbot[5] and the late H. M. Vango[6]

Abstract

Bovines fed B.C.G. shortly after birth do not show tuberculous lesions, nor any evidence of tuberculous infection at autopsy two years after such vaccination. This is demonstrated in animals vaccinated by mouth with suitable controls. Unvaccinated unprotected controls living closely in contact with these vaccinated animals were at the end of the same period quite free from tuberculous infection. Such evidence supports the contention that there is no return of virulence in B.C.G. in the animal body and that, therefore, vaccinated animals are not a source of danger to unprotected animals.

In a previous paper by the authors (3) attention was drawn to the resistance to tuberculous infection in animals vaccinated with B.C.G., and to the non-pathogenicity of B.C.G., grown according to Calmette's methods, in subcutaneously vaccinated calves over periods of one year.

It was also pointed out that animals vaccinated subcutaneously with B.C.G. exhibited a marked resistance to subsequent intravenous inoculations with virulent bovine bacilli, as compared with non-vaccinated controls, which confirmed the results of experiments carried out by Calmette and Guerin (2). These experiments were continued with the purpose of estimating the resistance to natural infection of calves vaccinated by mouth, and particularly of establishing the virulence or non-virulence of B.C.G. vaccine fed to new-born calves. The methods employed in the maintenance of the culture, preparation of the vaccine, etc., were those recommended by Calmette, and similar to those followed by the authors in their previous work (3). The culture used was received from Calmette in 1925, and since that time had been maintained on glycerinated potato and synthetic broth with occasional transfer to bile potato medium.

During August, September, and October 1928, 76 new-born calves were purchased in the neighborhood of Edmonton and brought together in a specially prepared barn on the outskirts of the city, where it was considered that they would be free from possible sources of tuberculous infection. These animals, with a few exceptions, were secured from dairy herds tested for tuberculosis,

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immediately after birth, in most cases even before they had been fed. They were, to the best of the authors' ability, protected from possible sources of tuberculous infection and were fed on pasteurized milk. Half of the number were vaccinated by mouth with three doses of B.C.G. vaccine (50 mg. moist weight) in milk on the third, fifth and seventh day subsequent to birth, the vaccine being composed of the bacilli from glycerinated potato or synthetic broth, and not more than 21 days old.

Mouth vaccination might be considered somewhat similar to such vaccination in children, though certainly not identical on anatomical and physiological grounds. As the organism is of bovine strain, its pathogenicity should manifest itself in the calf. The vaccinated animals and the controls though kept separated at first, in the barn and pasture, were under the same living conditions and were fed adequately for normal growth and maintenance*. During the months of September, October and November 1928, eight of these young animals died of disease other than tuberculosis, leaving at the end of the year, 68 animals, 34 of which had been vaccinated and 34 controls.

In March 1929, these animals were divided into the Edmonton and Ponoka groups, each consisting of 17 controls and 17 vaccinated animals. One group constituted the material for Experiment No. 1, 1928-30, an investigation of the virulence of the organism for new-born calves, and the other was used in Experiment No. 2, 1928-30, which was concerned with the resistance of calves vaccinated by mouth to contact infection. In the same month the animals of Experiment No. 2 were placed in contact with a tuberculous herd and fed on milk from tuberculous cows.

TABLE I

RESULTS OF EXPERIMENT NO. 1, 1928–1930, ON SIXTEEN QUARANTINED UNVACCINATED CONTROLS

No. of animal	Sex	Date of birth 1928	Date of slaughter 1930	Time elapsed, months	Post-mortem findings	
					Macroscopic	Microscopic
276	F	August 25	October 23	26	Negative	Negative
277	M	August 28	October 22	26	Negative	Negative
278	M	August 29	October 23	26	Negative	Negative
279	M	September 7	October 20	25	Negative	Negative
280	M	September 7	October 20	25	Negative	Negative
281	M	September 9	October 23	25	Negative	Negative
282	M	September 8	October 23	25	Negative	Negative
283	M	September 12	October 23	25	Negative	Negative
285	M	September 13	October 23	25	Negative	Negative
286	M	September 13	October 23	25	Negative	Negative
288	M	September 13	October 20	25	Negative	Negative
289	M	September 13	October 22	25	Negative	Negative
292	M	September 22	October 22	25	Negative	Negative
293	M	September 22	October 20	25	Negative	Negative
296	F	September 24	October 22	25	Negative	Negative
300	M	October 2	October 22	24	Negative	Negative

**On the advice of Professor J. P. Sackville, Professor of Animal Husbandry, University of Alberta.*

TABLE II

RESULTS OF EXPERIMENT NO. 1, 1928–30, ON SIXTEEN QUARANTINED ANIMALS VACCINATED BY MOUTH

No. of animal	Sex	Date of birth 1928	Date of vaccination 1928	Date of slaughter 1930	Time elapsed, months	Post-mortem findings	
						Macroscopic	Microscopic
251	M	August 17	August	October 20	26	Negative	Negative
252	M	August 15	August	October 22	26	Negative	Negative
253	M	August 22	August	October 20	26	Negative	Negative
254	M	August 24	August	October 20	26	Negative	Negative
255	M	August 26	August	October 20	26	Negative	Negative
258	M	August 29	August	October 20	25	Negative	Negative
259	F	August 29	August	October 20	25	Negative	Negative
261	M	August 30	August	October 20	25	Negative	Negative
263	M	August 30	August	October 20	25	Negative	Negative
264	M	September 1	August	October 20	25	Negative	Negative
265	M	September 1	August	October 20	25	Negative,	Negative
267	M	September 3	August	October 20	25	Negative	Negative
268	M	September 4	August	October 20	25	Negative	Negative
269	F	September 5	August	October 20	25	Negative	Negative
270	F	September 6	August	October 20	25	Negative	Negative
271	M	September 11	August	October 20	25	Negative	Negative

Investigation of Virulence

EXPERIMENT NO. 1, 1928-30

The Edmonton Group

In June 1929, animal No. 291 (a control) died of pneumonia, and in March 1930, animal No. 241 (vaccinated) was slaughtered because of its poor condition, leaving 32 animals in this experiment, 16 vaccinated and 16 controls.

In June 1929 the animals were allowed to run in a common pasture in which, as far as could be ascertained, there had been no animals for several years and which could, therefore, reasonably be considered to be non-infected. The pasture, which included bush, was enclosed by a wire fence to limit the movements of the animals to the non-infected area. Feed was supplied, the animals eating out of a common trough, and drinking Edmonton city water, also from a common trough. The controls and vaccinated animals therefore lived together, eating and drinking from the same supply of food and water, and being subject to the same possibilities of acquiring tuberculous infection. All feed, hay and grass were bought on the open market and did not come from protected sources.

On several occasions during the experiment, particularly in the spring of 1930, the animals broke through the retaining wire and were some hours at large in the neighborhood, where, however, there were no other cattle. These were, of course, breaks in the quarantine, but would not appear to have interfered with the experimental results as they have been subsequently recorded.

These animals passed the winter of 1929-30 together, mostly out of doors, and were at the time of slaughter over two years of age. They were slaughtered in Gainer's Packing Plant, Edmonton, on October 20-22 under careful observation, and while Federal Government inspectors were present. The whole

group of animals was passed for food, as there was no evidence of tuberculosis.

Glandular material taken at these autopsies was injected into guinea pigs and prepared for microscopic examination. In Tables I and II are shown the results of the examination of the tissues of these bovines.

The results of the microscopic examination of the glandular material from these animals confirm the macroscopic, and in the cases of control animals Nos 282 and 283, in which the macroscopic findings were slightly suspicious, demonstrated in one case actinomyces, and in the other a condition due to Gram positive branching filaments without spores or radial arrangement. Pigs inoculated with material from these lesions were negative to tests for tuberculosis. The tissues of the guinea pigs which were inoculated with glandular material from this group of animals on October 20 and 22, 1930, were examined both macroscopically and microscopically with negative results, and thus there was complete agreement between the three methods of examination. In Tables III and IV, are shown the results obtained.

The results of the experiment show that B.C.G. cultured and administered in the manner indicated is non-virulent for new-born calves. It is also evident that the passage of this organism through the vaccinated calves did not lead to the natural infection of the non-protected controls in close contact. Taken in conjunction with the authors' other experiments, already reported, we think we have very strong evidence indicating the non-virulence for bovines of this organism, carried according to Calmette's method, and we know of no publication in which the contrary is proved, experimentally or otherwise.

From the vaccinated animals of Experiment 2, 1928-30, we can be permitted, we think, to add 14 animals, vaccinated by mouth and which did not exhibit lesions attributable to B.C.G. vaccine, to the 16 mouth-vaccinated animals of this experiment, making in all 30 vaccinated animals quite free from tuberculous infection two years after mouth vaccination.

TABLE III

MACROSCOPIC AND MICROSCOPIC EXAMINATION OF GUINEA PIGS INOCULATED OCT. 20–22, 1930, WITH GLANDULAR MATERIAL FROM 16 QUARANTINED UNVACCINATED CONTROLS OF EDMONTON GROUP, EXPERIMENT 1

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
276	Pig 1	Died April 17, 1931. Acute gastroenteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	6
	Pig 2	Died April 21, 1931. Acute pneumonic consolidation—lungs. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	6
277	Pig 1	Died Nov. 16, 1930. Extensive pneumonic consolidation—both lungs. No evidence of tuberculosis. *Micro.*—Purulent pneumonia. No evidence of tuberculosis.	Not one month
	Pig 2	Died March 19, 1931. Extensive pneumonic lesions, acute pleurisy and pericarditis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5

TABLE III—*Continued*

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
278	Pig 1	Died Nov. 14, 1930. Pneumonic consolidation—lungs. No evidence of tuberculosis. *Micro.*—Acute pneumonia. No evidence of tuberculosis.	Not one month
	Pig 2	Died Nov. 19, 1930. Pneumonic lesions—lungs. No evidence of tuberculosis. *Micro.*—Purulent pneumonia. No evidence of tuberculosis.	Not one month
279	Pig 1	Died March 29, 1931. Acute pneumonia and peritonitis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
	Pig 2	Killed Aug. 20, 1931. Healthy pig. *Micro.*—No evidence of tuberculosis.	10
280	Pig 1	Died March 25, 1931. Recently pregnant. Pneumonic lesions in lung. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
	Pig 2	Killed Aug. 20, 1931. Healthy pig. *Micro.*—No evidence of tuberculosis.	10
281	Pig 1	Died Nov. 17, 1930. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	Not one month
	Pig 2	Died Feb. 12, 1931. Pneumonic consolidation of lung, empyema. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	3½
282	Pig 1	(Pus) Died Jan. 1, 1931. Extensive hemorrhagic pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	2
	Pig 2	(Pus) Killed March 30, 1931. Healthy pig. *Micro.*—No evidence of tuberculosis.	5
	Pig 3	Died Feb. 25, 1931. Gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4
	Pig 4	Died March 13, 1931. Extensive pneumonic lesions of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	4½
283	Pig 1	(Pus) Died Dec. 15, 1930. Extensive hemorrhagic pneumonic lesions of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1½
	Pig 2	(Pus) Died Feb. 17, 1931. Pig much emaciated. Recently pregnant. No gross evidence of disease. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	3
	Pig 3	Died April 21, 1931. Pig very thin. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—Not examined.	6
	Pig 4	Died July 7, 1931. Very thin, recently pregnant. No evidence of disease. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	8½

TABLE III—*Continued*

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
285	Pig 1	Died April 17, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	6
	Pig 2	Died April 21, 1931. Acute pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	6
286	Pig 1	Died Nov. 21, 1930. Extensive pneumonic consolidation of lung. Acute pleurisy. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	Not one month
	Pig 2	Died April 18, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	6
288	Pig 1	Died Jan. 16, 1931. Acute enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	2½
	Pig 2	Died March 8, 1931. Extensive pneumonic lesions of lungs. Empyema. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	3½
289	Pig 1	Died Nov. 22, 1930. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	Not one month
	Pig 2	Killed Aug. 20, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10
292	Pig 1	Died Jan. 12, 1931. Pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	2½
	Pig 2	Died Feb. 19, 1931. Pig very emaciated. Scattered pneumonic foci in lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	3
293	Pig 1	Died Feb. 22, 1931. No obvious cause of death. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4
	Pig 2	Died April 9, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
296	Pig 1	Died Dec. 12, 1930. Extensive hemorrhagic pneumonic lesion of lungs. Pig recently pregnant. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1½
	Pig 2	Died Feb. 23, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis,	4
300	Pig 1	Died Dec. 10, 1930. Extensive hemorrhagic pneumonic consolidation of lungs. Acute pleurisy. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1½
	Pig 2	Killed Aug. 27, 1931. Pregnant healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10

NOTE:—*Totals—16 animals, 36 guinea pigs. Portions of glands inoculated: submaxillary, pharyngeal, bronchial, mediastinal, portal, mesenteric, cervical. Epidemics of hemorrhagic pneumonia and gastro-enteritis in pigs.*

TABLE IV

MACROSCOPIC AND MICROSCOPIC EXAMINATION OF GUINEA PIGS INOCULATED OCT. 20–22, 1930 WITH GLANDULAR MATERIAL FROM SIXTEEN QUARANTINED ANIMALS VACCINATED BY MOUTH. EDMONTON GROUP, EXPERIMENT 1

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
251	Pig 1	Died Dec. 18, 1930. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	2
	Pig 2	Pig ill, killed June 29, 1931. No gross evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	8
252		Not examined.	
253	Pig 1	Died April 9, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
	Pig 2	Died June 2, 1931. A few congested areas in the lungs. No other abnormality. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	7½
254	Pig 1	Died Dec. 12, 1930. Recently pregnant. No gross pathological findings at autopsy. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	1½
	Pig 2	Died March 9, 1931. Acute enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4½
255	Pig 1	Died Jan. 19, 1931. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	3
	Pig 2	Killed Aug. 24, 1931. Recently pregnant, healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10
258	Pig 1	Died Feb. 2, 1931. Pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	3
	Pig 2	Died March 30, 1931. Recently pregnant pig. Totally exsanguinated (acute hemorrhage). No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
259	Pig 1	Died Dec. 18, 1930. Suppurative cervical lymphadenitis (smear shows Gram positive bacilli, no tubercle bacilli). Scattered pneumonic foci in lungs. No evidence of tuberculosis. *Micro.*—Suppurative cervical lymphadenitis. Acute bronchial pneumonia. No evidence of tuberculosis.	2
	Pig 2	Died Jan. 15, 1931. Extensive inflammatory lesions in subcutaneous and muscle planes. No evidence of tuberculosis. *Micro.*—Cellulitis (acute). No evidence of tuberculosis.	3
261	Pig 1	Died Nov. 22, 1931. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	13
	Pig 2	Died Feb. 26, 1931. Extensive pneumonic consolidation of lungs. Acute pericarditis. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. Acute pericarditis. No evidence of tuberculosis.	4

TABLE IV—*Continued*

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
263	Pig 1	Died Dec. 21, 1930. Recently pregnant. Acute pneumonic consolidation of lungs. Purulent pericarditis. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. Purulent pericarditis. No evidence of tuberculosis.	2
	Pig 2	Died April 13, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4½
264	Pig 1	Died Dec. 12, 1930. Extensive hemorrhagic pneumonic consolidation of lungs. Acute pleurisy. No evidence of tuberculosis. *Micro.*—Purulent bronchitis, pneumonia and pleurisy. No evidence of tuberculosis.	1½
	Pig 2	Died March 4, 1931. Recently pregnant. No gross pathology of organisms. *Micro.*—No evidence of tuberculosis.	4
265	Pig 1	Pig died Feb. 10, 1931. Extensive pneumonic lesions of lungs. Bilateral plastic pleurisy. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	3½
	Pig 2	Died April 19, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	6
267	Pig 1	Died Feb. 5, 1931. Acute pneumonic consolidation of lungs, with gangrene. No evidence of tuberculosis. *Micro.*—Purulent pneumonia, with gangrene. No evidence of tuberculosis.	3½
	Pig 2	Died March 30, 1931. Recently pregnant, marked pelvic peritonitis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
268	Pig 1	Died Dec. 12, 1930. Extensive pneumonia hemorrhagic lesions of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1½
	Pig 2	Died March 15, 1931. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4½
269	Pig 1	Died Nov. 22, 1930. Extensive hemorrhagic pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Extensive purulent pneumonia. No evidence of tuberculosis.	1
	Pig 2	Died March 6, 1931. Recently pregnant. Acute endometritis. Broncial pneumonia. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4½
270	Pig 1	Died Nov. 18, 1930. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1
	Pig 2	Died Feb. 27, 1931. Pneumonic consolidation of lungs. Recently pregnant. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	4
271	Pig 1	Died Jan. 19, 1931. Pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	3
	Pig 2	Died Feb. 26, 1931. Extensive pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4

NOTE:—*Totals—16 animals, 30 guinea pigs. Portions of glands inoculated: submaxillary, pharyngeal, bronchial, mediastinal, portal, mesenteric, cervical. Epidemics of pneumonia and gastro-enteritis in pigs.*

Investigation of the Resistance to Contact Infection of Animals Vaccinated by Mouth

Ponoka Group

EXPERIMENT No. 2, 1928-30

As stated, the animals of this experiment were, in March 1929, placed in contact with a tuberculous herd. For a period of four months they were

TABLE V

RESULTS OF EXAMINATION OF 16 UNVACCINATED CONTROLS PLACED IN CONTACT WITH TUBERCULOUS ANIMALS. PONOKA GROUP, EXPERIMENT 2, 1928–30.

No. of animal	Sex	Date of birth 1928	Placed in contact 1929	Time in contact in months	Date of slaughter 1930	Age at slaughter in months	Post-mortem findings	
							Macroscopic	Microscopic
226	M	Sept. 6	March	6+	Oct. 31	25		
227	M	Sept. 7	March	6+	Oct. 31	25		
228	M	Sept. 9	March	6+	Oct. 31	25		Positive
229	M	Sept. 11	March	6+	Oct. 31	25		
231	M	Sept. 12	March	6+	Oct. 31	25	Positive	Positive
233	F	Sept. 13	March	6+	Oct. 31	25		
234	M	Sept. 14	March	6+	Oct. 31	25		
235	F	Sept. 14	March	6+	Nov. 1	25	Positive	Positive
236	F	Sept. 15	March	6+	Nov. 1	25		
237	M	Sept. 17	March	6+	Nov. 1	25	Positive	Positive
238	M	Sept. 18	March	6+	Nov. 1	25		
239	M	Sept. 18	March	6+	Nov. 1	25	Positive	Positive
295	M	Aug. 24	March	6+	Nov. 1	26	Positive	Positive
297	M	Aug. 24	March	6+	Nov. 1	26		
298	M	Aug. 24	March	6+	Nov. 1	26		
299	M	Aug. 25	March	6+	Nov. 1	26		

NOTE:—*Total, 16; positive 6.*

TABLE VI

RESULTS OF EXAMINATION OF 17 ANIMALS VACCINATED BY MOUTH AND PLACED IN CONTACT WITH TUBERCULOUS ANIMALS. PONOKA GROUP, EXPERIMENT 2, 1928–30

No. of animal	Sex	Date of birth 1928	Date of vacci-nation 1928	Placed in contact 1929	Time in contact in months	Date of slaughter 1930	Age at slaughter in months	Post-mortem findings	
								Macroscopic	Microscopic
242	M	Oct. 3	Oct.	March	6+	Oct. 31	25	Positive	Positive
243	M	Oct. 1	Oct.	March	6+	Oct. 31	25		
245	M	Oct. 1	Oct.	March	6+	Oct. 31	25		
246	M	Oct. 1	Oct.	March	6+	Oct. 31	25		
247	M	Sept. 29	Sept.	March	6+	Oct. 31	25		
248	M	Sept. 28	Sept.	March	6+	Oct. 31	25		
249	M	Sept. 27	Sept.	March	6+	Oct. 31	25	Positive	Positive
257	M	Sept. 21	Sept.	March	6+	Oct. 31	25		
272	M	Sept. 11	Sept.	March	6+	Nov. 1	25		
273	M	Sept. 19	Sept.	March	6+	Nov. 1	25		
274	M	Sept. 19	Sept.	March	6+	Nov. 1	25		
275	M	Sept. 20	Sept.	March	6+	Nov. 1	25		
303	M	Oct. 19	Oct.	March	6+	Nov. 1	24		
304	M	Oct. 20	Oct.	March	6+	Nov. 1	24		
305	M	Oct. 20	Oct.	March	6+	Nov. 1	24	Positive	Positive
307	F	Oct. 22	Oct.	March	6+	Nov. 1	24		
308	F	Oct. 28	Oct.	March	6+	Nov. 1	24		

NOTE:—*Total, 17; positive, 3.*

TABLE VII

MACROSCOPIC AND MICROSCOPIC EXAMINATION OF GUINEA PIGS INOCULATED ON OCTOBER 31 AND NOVEMBER 1, 1930 WITH GLANDULAR MATERIAL FROM 16 UNVACCINATED CONTROLS IN CONTACT WITH TUBERCULOUS ANIMALS. PONOKA GROUP, EXPERIMENT 2

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
226	Pig 1	Died April 10, 1931. Pneumonic lesions of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	5½
	Pig 2	Killed Oct. 10, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	11½
227	Pig 1	Died April 13, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
	Pig 2	Died April 15, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
228	Pig 1	Died May 2, 1931. Generalized tuberculosis. *Micro.*—Generalized tuberculosis.	
	Pig 2	Died May 21, 1931. Generalized tuberculosis. *Micro.*—Generalized tuberculosis.	
229	Pig 1	Died April 13, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
	Pig 2	Died April 16, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
231	Pig 1	Died Jan. 25, 1931. Tuberculosis of superficial and deep inguinal nodes, lungs, liver and spleen.	
	Pig 2	Died Feb. 12, 1931. Tuberculosis of superficial and deep inguinal nodes, lungs, liver and spleen.	
233	Pig 1	Killed Sept. 14, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10½
	Pig 2	Killed Sept. 19, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10½
234	Pig 1	Died Dec. 2, 1930. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	1
	Pig 2	Died March 10, 1931. Acute enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4
235	Pig 1	Died Feb. 24, 1931. Extensive generalized tuberculosis. *Micro.*—Extensive generalized tuberculosis.	
	Pig 2	Died April 15, 1931. Extensive generalized tuberculosis.	
236	Pig 1	Died April 25, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
	Pig 2	Killed Aug. 13, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	9
237		No pigs inoculated.	

TABLE VII—*Continued*

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
238	Pig 1	Died April 15, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
	Pig 2	Died April 17, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
239	Pig 1	Killed Sept. 19, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10½
	Pig 2	Killed Sept. 19, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10½
295	Pig 1	Died Dec. 25, 1930. Extensive generalized tuberculosis.	
	Pig 2	Died Dec. 26, 1930. Extensive generalized tuberculosis.	
297	Pig 1	Died Nov. 24, 1930. Extensive hemorrhagic pneumonic consolidation of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	Not one month
	Pig 2	Died March 3, 1931. Extensive pneumonic consolidation of lungs. Acute pleuritis, pericarditis, peritonitis. Recently pregnant. No evidence of tuberculosis. *Micro.*—Generalized septico-pyaemia. No evidence of tuberculosis.	4
298	Pig 1	Died Dec. 11, 1930. Pneumonic lesions of lungs. Recently pregnant. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1
	Pig 2	Died March 10, 1931. Pneumonic lesions of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	4
299	Pig 1	Died April 15, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5½
	Pig 2	Killed Aug. 27, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	9½

NOTE:—*Totals—16 animals, 30 guinea pigs. Portions of glands inoculated: submaxillary, pharyngeal, bronchial, mediastinal, portal, mesenteric, cervical. Epidemics of pneumonia and gastro-enteritis in pigs. Animal No. 228, not macroscopically positive, see Table No. IV.*

fed with milk from tuberculous cattle and on April 10 were placed in close contact with tuberculous cows. This contact was maintained latterly in the open, till November 1, 1929, when they were removed from contact. They passed the winter of 1929-30 in a straw shed, which had been previously used for tuberculous experimental cattle. During the following summer they were again in the open until the time of slaughter. A control animal, No. 232,

TABLE VIII

MACROSCOPIC AND MICROSCOPIC EXAMINATION OF GUINEA PIGS INOCULATED OCTOBER 31 AND NOVEMBER 1, 1930 WITH GLANDULAR MATERIAL FROM 17 ANIMALS VACCINATED BY MOUTH AND PLACED IN CONTACT WITH TUBERCULOUS ANIMALS. PONOKA GROUP, EXPERIMENT 2

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
242	Pig 1	Died April 30, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	6
	Pig 2	Killed Sept. 26, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10½
243	Pig 1	Died April 13, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
	Pig 2	Killed Oct. 29, 1931. No evidence of tuberculosis.	12
245	Pig 1	Killed Aug. 17, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	9½
	Pig 2	Killed Aug. 17, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	9½
246	Pig 1	Died March 19, 1931. Acute enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	4½
	Pig 2	Killed Oct. 10, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	11
247	Pig 1	Died April 9, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
	Pig 2	Killed Oct. 19, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	11½
248	Pig 1	Died Nov. 29, 1930. Pneumonic lesions of lungs. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1
	Pig 2	Killed Oct. 22, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	11½
249	Pig 1	Died Dec. 19, 1930. Extensive generalized tuberculosis.	
	Pig 2	Died Dec. 27, 1930. Extensive generalized tuberculosis.	
	Pig 3	(Reinoculated from Pig 2). Killed Feb. 24, 1931. Extensive generalized tuberculosis.	
	Pig 4	(Subinoculated from Pig 3). Died March 30, 1931. Extensive generalized tuberculosis.	
257	Pig 1	Died April 9, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
	Pig 2	Killed Oct. 29, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	12
272	Pig	No record.	
	Pig 2	Killed Sept. 14, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10

TABLE VIII—*Continued*

No. of animal	Guinea pigs inoculated	Details of post-mortem findings	Time elapsed in months
273	Pig 1	Died April 19, 1931. Acute gastro-enteritis. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
	Pig 2	Killed Aug. 17, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	9
274	Pig 1	Died April 9, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
	Pig 2	Killed Oct. 19, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	11
275	Pig 1	Died Nov. 29, 1930. No evidence of tuberculosis. *Micro.*—Acute bronchial pneumonia. No evidence of tuberculosis.	1
	Pig 2	Died April 7, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	5
303	Pig 1	Died Nov. 28, 1930. Pneumonic foci in lungs. No evidence of tuberculosis. *Micro.*—Bronchial pneumonia. No evidence of tuberculosis.	1
	Pig 2	Died Nov. 28, 1930. Acute purulent pneumonia. No evidence of tuberculosis. *Micro.*—Acute purulent pneumonia. No evidence of tuberculosis.	1
304	Pig 1	No record.	
	Pig 2	Killed Oct. 22, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	11
305	Pig 1	Died Dec. 31, 1930. Extensive generalized tuberculosis.	
	Pig 2	Died Jan. 27, 1931. Extensive generalized tuberculosis.	
307	Pig 1	Killed Sept. 14, 1931. Pregnant healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	10
	Pig 2	Killed Oct. 10, 1931. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	11
308	Pig 1	Died April 18, 1931. Acute gastro-enteritis. No evidence of tuberculosis.	5
	Pig 2	Killed Aug. 17, 1931. Healthy pig. No evidence of tuberculosis. *Micro.*—No evidence of tuberculosis.	9

NOTE:—*Totals—17 animals, 36 guinea pigs. Portions of glands inoculated: submaxillary, pharyngeal, bronchial, mediastinal, portal, mesenteric, cervical. Epidemics of pneumonia and gastro-enteritis in pigs. Animal No. 242—the small gastric tuberculous gland, upon which the autopsy diagnosis was based, was not inoculated into pigs, although the macroscopic finding was confirmed microscopically.*

which was in poor condition, and finally became mired, was found dead on September 16, 1929. Post-mortem examination did not show evidence of tuberculosis. These animals, now reduced to thirty-three, seventeen vac-

cinated and sixteen controls, were slaughtered on October 31 and November 1, 1930, in the newly erected slaughter house at Ponoka Institute, and examined for the presence of tuberculous lesions. Guinea pigs were inoculated with glandular material and tissue was taken for microscopic examination.

At the time of slaughter these animals were over two years of age, and had been in contact with tuberculous animals, commencing nineteen months previously, for a period of more than six months. The vaccinated animals had been vaccinated five months previous to contact so that, at the termination of contact, over eleven months had passed since vaccination, and it was for this reason that contact was discontinued on November 1, 1929.

The results of this experiment perhaps indicate an increased resistance in the vaccinated animals—80% free from tuberculosis as compared to 60% in the controls. The lesions found were slight; glandular only in the three infected vaccinated animals, microscopic glandular lesions in one of the six infected controls, and organic lesions in only one. The chances of natural infection would appear not to have been as great as desired. These results are confirmed by microscopic examination of glandular material. As in the previous experiment the results of the macroscopic and microscopic examination of the tissues of the inoculated guinea pigs correspond to similar examination of the tissues of the bovines*. The detailed results of the examinations are given in Tables V, VI, VII, VIII, IX and X.

The experiment is interesting from the point of view of methods employed for the maintenance of quarantine and might be taken to illustrate the possibilities of tuberculous infection in young bovines so protected. We think also that it reflects creditably upon similar arrangements—used in our previously

TABLE IX

AUTOPSY FINDINGS (MACROSCOPIC AND MICROSCOPIC) ON SIXTEEN UNVACCINATED CONTROLS OF WHICH SIX SHOWED TUBERCULOUS LESIONS, EXPERIMENT 2, 1928–30

No. of animal	Nature and extent of post-mortem findings	Results of guinea pig inoculation
228	Portal gland shows, microscopically, scattered conglomerate tubercles not visible macroscopically.	Positive
231	One peri-bronchial lymph node shows small caseo-calcareous lesion. Lungs, small caseo-calcareous lesion.	Positive
235	Retro-pharyngeal lymph nodes show extensive caseous lesions.	Positive
237	One calcified mesenteric lymph node.	No test
239	Portal lymph node caseous.	No test, other glands negative
295	One mesenteric gland calcareous.	Positive
All others	Negative.	Negative

**With respect to animals Nos. 237 and 239 the single tuberculosis gland found in each case was not inoculated into guinea pigs.*

TABLE X

AUTOPSY FINDINGS (MACROSCOPIC AND MICROSCOPIC) ON SEVENTEEN VACCINATED ANIMALS, THREE OF WHICH SHOWED TUBERCULOUS LESIONS. EXPERIMENT 2, 1928-30

No. of animal	Nature and extent of post-mortem findings	Results of guinea-pig inoculation
242	One gastric lymph node showed a microscopic caseo-calcareous lesion. Macroscopically suspicious.	No test. All other glands negative
249	One of the retro-pharyngeal nodes caseo-calcareous.	Positive
305	One caseous mesenteric lymph node.	Positive
All others	Negative.	Negative

published experiments—for the exclusion from our experimental animals of tuberculous infection from outside sources. We are of the opinion that the duration of the experiment, which was over two years, strengthens any conclusions that seem justifiable.

Conclusions

1. B.C.G. vaccine is non-pathogenic for bovines.
2. Mouth vaccination with B.C.G. probably produces moderate resistance in bovines.
3. It would appear that new-born calves vaccinated by mouth are not capable of subsequently transmitting infection to non-vaccinated controls in close contact, and that vaccination is, therefore, no menace to non-protected animals.
4. B.C.G. does not produce bovine carriers of virulent tubercle bacilli.
5. As B.C.G. is a bovine strain, and has been shown to be non-pathogenic for young calves—the natural host—we think it may be assumed that, carried according to Calmette's method, this organism is non-virulent and will not dissociate or revert to type in the tissues.

Acknowledgments

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References

1. BUXTON, J. B. and GRIFFITH, A. S. Lancet, 220: 393-401. 1931.
2. CALMETTE, A. and GUERIN, C. Ann. inst. Pasteur, 38: 371-398. 1924.
3. RANKIN, A. C. Can. J. Research, 1: 48-85. 1929.

DISPERSION AND SELECTIVE ABSORPTION IN THE PROPAGATION OF ULTRASOUND IN LIQUIDS CONTAINED IN TUBES[1]

PART II

By G. S. Field[2] and R. W. Boyle[3]

Abstract

More experimental work has been done on the propagation of ultrasound along tubes filled with liquid. The mathematical theory developed by Field has been subjected to a quantitative verification, and it has been shown that the discontinuities in the velocity-frequency curve are due to a transference of energy at certain frequencies from the longitudinal to a radial vibration. It has also been shown that the theory satisfactorily accounts for the observed facts, the phase velocities, particle velocities and the absorption frequencies having the values which the theory requires.

Introduction

Following the experimental work of Boyle, Froman and Field (1), and the* development of a formula by Field (3), to explain the experimental observations, it was decided to carry out a further research in order to subject the mathematical theory of the transference of energy to a radial vibration to a quantitative verification.

Accordingly, three separate experiments were undertaken, these being (1) a determination and comparison of observed with calculated phase velocities, over a range of frequencies involving two absorption bands; (2) a determination of the frequencies of five absorption bands in a tube of liquid and a comparison of the observed with the theoretical frequencies; and finally (3) a determination of radial particle velocities in a tube of liquid at a lower and a higher frequency than that of the first absorption band, and a comparison of these velocities with their calculated values from the theory.

Phase Velocities

To measure velocities of sound in tubes, the final method adopted in Part I was employed. That is to say, a rod-oscillator was fitted into each end of a glass tube, cavitation was produced in the liquid, and the distance between a number of nodes, as indicated by rising curtains of bubbles, was measured. Knowing the frequency of the electrical oscillator, the phase velocity of ultrasound in the liquid was then easily determined. In this way, velocities over a range of frequencies were obtained for naphtha in two different sizes of glass tubes. The results are plotted graphically in Figs. 1 and 2.

For the theoretical calculations, the following formulas were employed,

$$x\frac{J_1(x)}{J_0(x)} = \frac{\dfrac{-a}{h}}{\dfrac{E'}{a^2\omega^2\rho} - \dfrac{\rho_1}{\rho}} \tag{1}$$

Reference 3, p. 142, formula 48.

[1] *Manuscript received January 16, 1931.*
Contribution from the National Research Laboratories, Ottawa, Canada.

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$$x' \frac{J_1(x')}{J_0(x')} = \frac{\frac{+a}{h}}{\frac{E'}{a^2\omega^2\rho} - \frac{\rho_1}{\rho}} \qquad (2)$$

In the case of the first tube, the physical constants were: $a=0.97$; $h=0.13$; $\rho=0.74$; $\rho_1=2.6$; $E=6.03\times10^{11}$; $c_0=1.26\times10^5$.

$$E' = \frac{E}{1-\frac{5h}{6a}} = 6.78\times10^{11}.$$

Substituting the numerical values in Equation 1 gives,

$$x\frac{J_1(x)}{J_0(x)} = \frac{-1}{\frac{33.2\times10^8}{n^2}-0.47} \qquad (3)$$

Each value of n ($n>n_0$, where n_0 = absorbing frequency) determines a value of $x\frac{J_1(x)}{J_0(x)}$, and hence of x. By tabulating a few values of $x\frac{J_1(x)}{J_0(x)}$ it is possible to fix x approximately, and trial and error will then readily give x to one or two decimal places. There is no particular point in determining x very exactly, as the calculations in any case involve physical constants whose values are known only approximately.

TABLE I

THEORETICAL VELOCITY CALCULATIONS FOR TUBE 0.97 CM. IN RADIUS, FOR FREQUENCIES *above* ABSORPTION BAND

N in kilo-cycles/sec.	$X\frac{J_1(x)}{J_0(x)}$	X	$N_x=\frac{(xc)}{(2\pi a)}$	$N^2\times10^{-8}$	$N_x^2\times10^{-8}$	$(\sqrt{N^2-N_x^2})\times10^{-4}$	$C_1\times10^{-5}$
65	− 3.1	3.12	64.5	42.3	41.6	0.84	9.75
70	− 4.8	2.90	59.9	49	35.9	3.62	2.44
80	−20.1	2.53	52.3	64	27.4	6.02	1.67
90	+16.7	2.26	46.7	81	21.8	7.69	1.48
100	+ 7.1	2.10	43.4	100	18.8	9.01	1.40
110	+ 5.0	1.99	41.2	121	17.0	10.2	1.36
120	+ 4.2	1.93	39.9	144	15.9	11.3	1.34

TABLE II

THEORETICAL VELOCITY CALCULATIONS FOR TUBE 0.97 CM. IN RADIUS, FOR FREQUENCIES *below* ABSORPTION BAND

N in kilo-cycles/sec.	$X^1\frac{I_1(x^1)}{I_0(x^1)}$	X^1	$N_x^1=\frac{(x^1C)}{(2\pi a)}$	$N^2\times10^{-8}$	$N_{x'}^2\times10^{-8}$	$(\sqrt{N^2\times N_{x'}^2})\times10^{-4}$	$C_1\times10^{-5}$
30	0.311	0.82	17	9	2.89	3.45	1.10
40	0.625	1.21	25	16	6.25	4.72	1.07
50	1.16	1.80	37.2	25	13.8	6.23	1.01
60	2.22	2.80	57.9	36	33.5	8.34	0.91
70	4.77	5.2	108	49	116.6	12.9	0.68
80	20	2.1	434	64	1884	44.1	0.23
84	∞	∞	∞	70.6	∞	∞	0

**Reference 3, p. 143, formula 59.*

The results of calculations of phase velocity for a number of frequencies are shown in Table I and plotted in Fig. 1.

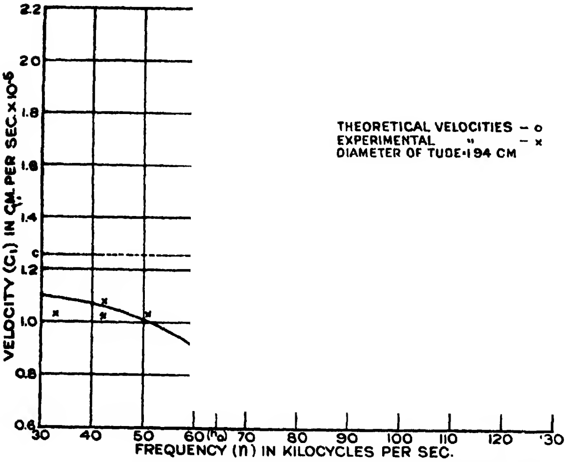


FIG. 1. *Comparison of theoretical and experimental phase velocities for naphtha in a glass tube, 1.94 cm. diameter and 1.2 mm. wall thickness.*

For low frequencies it is necessary to use Equation 2. When the numerical values are substituted, an equation similar to Equation 3 is obtained. Values of x' are determined as above. Using these values, phase velocities were calculated for frequencies below the first absorption band. The results are shown in Table II and plotted in Fig. I.

For the second tube, $a = 1.55$; $h = 0.14$; $c_0 = 1.21 \times 10^5$ (different sample of lighting naphtha); other constants as before.

In this case we have for high frequencies,

$$x \frac{J_1(x)}{J_0(x)} = \frac{-1}{\dfrac{8.76 \times 10^8}{n^2} - 0.316} \tag{4}$$

And for low frequencies,

$$x' \frac{I_1(x')}{I_0(x')} = \frac{1}{\dfrac{8.76 \times 10^8}{n^2} - 0.316} \tag{5}$$

Calculations of velocity were made from these two formulas and are shown in Tables III and IV. The theoretical curve resulting therefrom is shown in Fig. 2.

In order to show the effect of radial resonance for modes other than the fundamental, a calculation was made of a few velocities at frequencies just above that of the first overtone on the assumption that resonance was occurring at this frequency.

Mathematically, this means simply taking the second solutions for x, and carrying through the calculations therewith. Table V shows the results of these computations and in Fig. 2 the theoretical velocities are plotted.

Remarks

The agreement between calculated and observed values of velocity on the high frequency side of the absorption band is seen to be very good. On the low frequency side the theoretical velocities are too low in the immediate vicinity of the absorbing frequency. This was expected, however, and an explanation was given in the theoretical paper (3), where it was suggested that if viscosity were included in the analysis, better agreement would most likely be obtained.

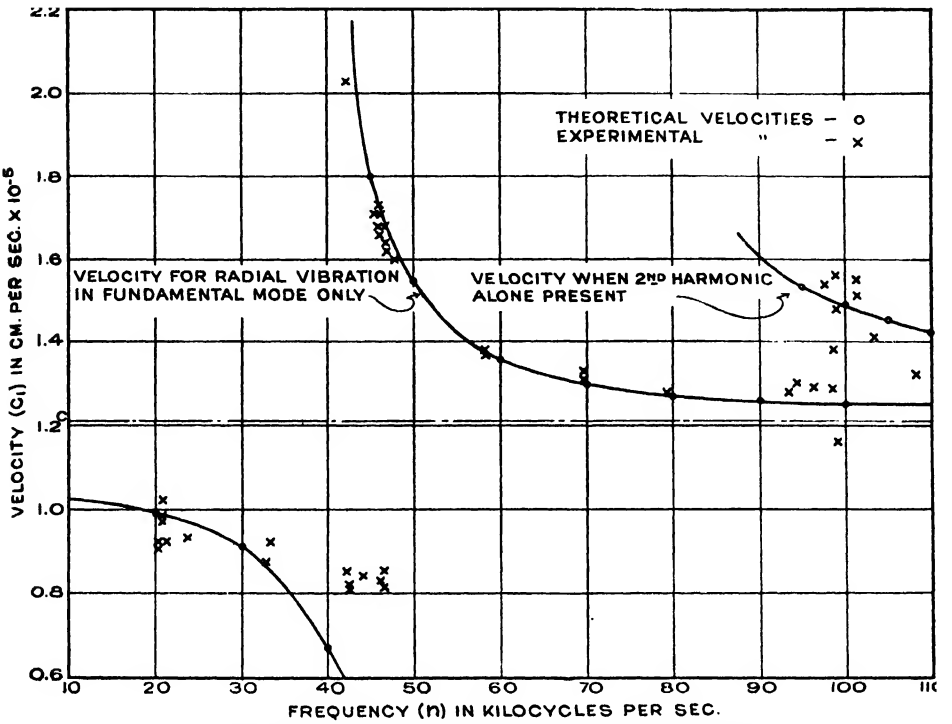


FIG. 2. *Comparison of theoretical and experimental phase velocities for naphtha in a glass tube, 3.1 cm. diameter and 1.4 mm. wall thickness.*

TABLE III

THEORETICAL VELOCITY CALCULATIONS FOR TUBE 1.55 CM. IN RADIUS, FOR FREQUENCIES *above* FIRST ABSORPTION BAND

N in kilo-cycles/sec.	$X\dfrac{J_1(x)}{J_2(x)}$	X	$N_x=\left\{\dfrac{CX}{2\pi a}\right\}$	$N^2\times10^{-8}$	$N^2\times10^{-8}$	$(\sqrt{N^2-N_x^2})\times10^{-4}$	$C_1\times10^{-5}$
100	4.38	1.94	24.1	100	5.81	9.71	1.25
90	4.81	1.97	24.5	81	6.00	8.66	1.26
80	5.58	2.03	25.2	64	6.35	7.59	1.28
70	7.30	2.10	26.1	49	6.81	6.50	1.30
60	13.7	2.23	27.7	36	7.67	5.32	1.36
50	−29.4	2.50	31.1	25	9.67	3.91	1.55
45	−8.62	2.70	33.5	20.3	11.22	3.02	1.80
40	−4.33	2.97	36.9	16	13.62	1.55	3.12

At high frequencies, where first overtone resonance is beginning to occur, the phase velocities become indefinite. The theoretical curves suggest that either one or both of two different velocities may occur, and the experimental readings indicate that the actual velocity has a definite value somewhere between the two, except at about 99 kilocycles, where the velocity may be anything between the two. This seems to be a sort of change-over point,

TABLE IV

THEORETICAL VELOCITY CALCULATIONS FOR TUBE 1.55 CM. RADIUS, FOR FREQUENCIES *below* FIRST ABSORPTION BAND

N in kilo-cycles/sec.	$X\frac{I_1(x^1)}{I_o(x^1)}$	X^1	$N_{x'}=\left(\frac{CX^1}{2\pi a}\right)$	$N^2\times10^{-8}$	$N_{x'}^2\times10^{-8}$	$(\sqrt{N^2+N_{x'}^2})\times10^{-4}$	$C_1\times10^{-5}$
20	0.534	1.11	13.8	4	1.90	2.43	0.99
30	1.54	2.14	26.6	9	7.08	4.01	0.91
40	4.32	4.88	60.6	16	36.7	7.26	0.67
50	29.4	30	373	25	1390	37.6	0.16

TABLE V

THEORETICAL VELOCITY CALCULATIONS FOR TUBE 1.55 CM. IN RADIUS FOR FREQUENCIES *above second* ABSORPTION BAND

N in kilo-cycles/sec.	$X\frac{J_1(X)}{J_o(x)}$	X	$N_x=\left(\frac{CX}{2\pi a}\right)$	$N^2\times10^{-8}$	$N_x^2\times10^{-8}$	$(\sqrt{N^2-N_x^2})\times10^{-4}$	$C_1\times10^{-5}$
120	3.92	4.59	57	144	32.5	10.6	1.37
110	4.09	4.61	57.3	121	32.8	9.39	1.42
105	4.22	4.63	57.5	110	33.1	8.77	1.45
100	4.38	4.65	57.8	100	33.4	8.16	1.49
95	4.57	4.67	58.0	90.3	33.6	7.53	1.53

where the velocity goes from one type of vibration to the other. This corresponds to the frequency 45 kilocycles, where two distinct groups of velocities were obtained, one group with an average high value and the other with a low.

Absorption of Sound Energy in the Tube of Liquid

To measure the absorption of energy in a tube containing liquid, apparatus was set up as shown in Fig. 3. A and B are two galvanized iron tanks, with an arrangement at one end for admitting a glass tube (Tank B) and a piston oscillator (Tank A) and for making water-tight connections therewith. T_c and T_t are torsion pendula with their accompanying torsion heads and telescopes, similar to apparatus previously used in ultrasonic energy measurements. The pendula used in this research were the single "air" vane type (2).

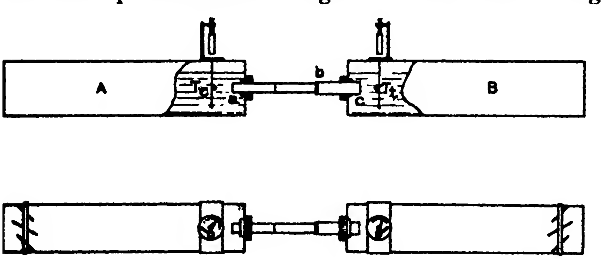


FIG. 3. *Apparatus used for experimentally determining the proportion of energy transmitted along a tube at different frequencies.*

To measure the absorption in the tube of liquid, the most direct way would be to measure the energy of the sound at b and c. The difference between the two energy measurements would then represent the amount absorbed by the

cylinder of liquid between *b* and *c*. It is, however, almost impossible to measure energies reliably inside a tube, such as at *b*, so that it was necessary to employ an indirect method which would give equivalent results. In this case, the difficulty was overcome by taking readings at *a*. In doing this it was tacitly assumed that the amplitude of vibration of the oscillator at *a* was equal to the amplitude at *b*. This was probably not entirely true, but since each end was vibrating in the same liquid and each was clamped with a rubber washer (one at the tank and one at the glass tube), the difference should not have been great. The experimental results appeared to justify the assumption made.

It was also assumed that the energy field about the end of the rod at *a* varied with frequency in about the same manner as the field about the end of the tube at *c*, because at the frequencies used no interference phenomena could intervene, such as the formation of a beam of ultrasound from the ends of the oscillator or tube, or the production of any regions of maximum or minimum energy intensity along the axis. Hence deceptive readings of the measuring pendula could not occur.

Let P_c = deflection of pendulum T_c, and P_t = deflection of pendulum T_t.

Then $K\dfrac{P_t}{P_c}$ = proportion of energy transmitted through tube, where K = a constant, which would equal unity if the energy intensities at *a* and *b* were exactly similar, and if the two pendula were exactly the same.

By plotting $\dfrac{P_t}{P_c}$ against frequency, therefore, we obtain a curve of relative transmission at the different frequencies.

In order to obtain reasonable readings with the torsion pendula, it was necessary to operate the rod oscillator at a resonance point. This meant that any slight variation in frequency caused a large change in radiated energy and hence a large variation in pendula readings. After a number of vain attempts to keep the frequency sufficiently constant to obviate the difficulty, it was found that fairly consistent results could be obtained by watching each pendulum in turn and taking the maximum readings. Three or four such readings were taken in each case, and the average noted.

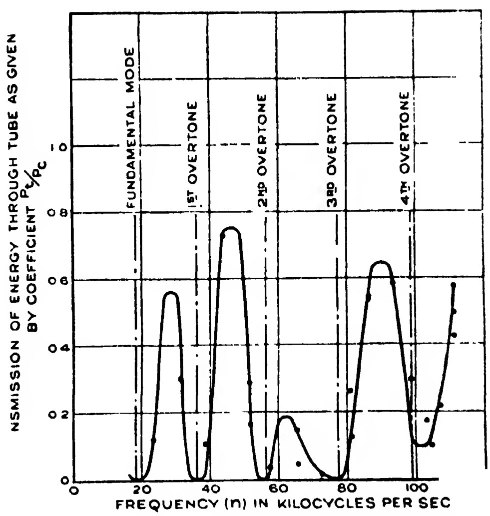


FIG. 4. *Curve showing the absorption bands occurring for energy transmitted through water contained in a glass tube.*

Since several different suspensions were used, on account of breakages, it was necessary to correlate the different sets of readings to allow for different

values of K. This was done by comparing deflections taken at the same frequency, but with the different suspensions. Care was taken that the frequency used for correlating readings was one which corresponded to large pendula deflections, since these readings would most likely have the smallest percentage error and for these frequencies the absorption had minimum values. The experimental results are given in Table VI and plotted in Fig. 4.

In order to compare the observations with the theory, the frequencies of radial resonance were calculated using the formulas,

$$\frac{J_1(x)}{J_0(x)} = \frac{-xac^2\rho}{h(E'-x^2\bar{c}^2\rho_1)} \; * \tag{6}$$

$$n = \frac{cx}{2\pi a} \; ** \tag{7}$$

In this case, $a=3.38$, $h=0.18$, $c=1.52\times10^5$, $E'=6.78\times10^{11}$, $\rho_1=2.7$, $\rho=1.0$.

Hence,

$$\frac{J_1(x)}{J_0(x)} = \frac{-x}{1.6-0.144x^2} \tag{8}$$

$$n = \frac{cx}{2\pi a} \tag{9}$$

From these equations the frequencies of radial resonance were calculated to be as in Table VII, and indicated on the absorption curve by vertical lines.

TABLE VI

EXPERIMENTAL DETERMINATION OF ABSORPTION AND TRANSMISSION OF SOUND ALONG A TUBE CONTAINING WATER

Frequencies in kilocycles	P_c deflection in deg.	P_t deflection in deg.	$\frac{P_t}{P_c}$	Correlated $\frac{P_t}{P_c}$
65.6	105	5	0.05	0.05
72.5	99	0	0.00	0.00
81.0	53	14	0.26	0.26
86.8	62	34	0.55	0.55
93.7	117	69	0.59	0.59*
51.6	27	4½	0.17	0.17
65.5	89	13	0.15	0.15
72.5	139	3	0.02	0.02
81.3	64	8	0.13	0.13
86.2	31	24	0.77	0.53
93.6	44	38	0.86	0.59*
99.0	39	17	0.44	0.30†
58.3	61	7	0.11	0.08
57.3	47	3	0.06	0.04
51.6	24	10	0.42	0.29
43.5	14	15	1.07	0.73
38.3	166	26	0.16	0.11
31.0	50	22	0.44	0.30
23.1	45	8	0.18	0.12
99.0	73	28	0.38	0.30†
97.8	30	15	0.50	0.39
103	30	7	0.23	0.18
104.7	40	5	0.13	0.10
106.6	37	10	0.27	0.21
111.2	{110	59	0.54	0.43
	{66	49	0.74	0.58
111.7	114	73	0.64	0.51

*Readings correlating groups 1 and 2. †Readings correlating groups 2 and 3.

*Reference 3, p. 138, formula 22.
**Reference 3, p. 138, formula 23.

TABLE VII

FREQUENCIES OF RADIAL RESONANCE IN TUBE OF WATER, AS CALCULATED FROM THEORY

Mode of vibration	Fundamental	1st overtone	2nd overtone	3rd overtone	4th overtone
Frequency, cycles/sec.	18,800	36,600	56,400	77,400	99,000

Radial Particle Velocities

In attempting to measure radial particle velocities in the tube, it was hoped to show three things: first, that a radial vibration was actually occurring at certain frequencies in the liquid even though presumably only a longitudinal wave was started at one end; second, that the type of vibration above the frequency of the first absorption band was different from the type at a frequency below it; and third, that the particle velocities were substantially as indicated by the theory.

To measure these particle velocities, a new experiment was devised making use of the theory of the Rayleigh disk. A tube of water was mounted vertically and set into vibration by a rod oscillator fitted with a rubber gasket to the lower end. A Rayleigh disk (0.8 cm. in diameter) was made of copper sheet, 0.47 mm. thick, and suspended in the liquid by a single silk fibre from a suitable torsion head. The reason for using the silk fibre was that the turning moment produced by the movement of the particles of liquid was very slight, and other suspensions (*e.g.*, a very thin gold wire) which were tried were found to be too stiff to give a good angle of twist.

The disk was viewed with a telescope and set approximately at 45° to a radius vector. The oscillator was started and the tendency of the disk to rotate, caused by the action upon it of the particles of liquid, was compensated by twisting the fibre suspension at the torsion head.

The twisting moment on the disk caused by the moving liquid is given by the relation,

$$M = K\dot{\zeta}^2 \sin 2\varphi \quad * \tag{10}$$

Where M = twisting moment, K = a constant, $\dot{\zeta}$ = particle velocity, φ = angle between the normal to the disk and the direction of the undisturbed stream.

Since the disk was kept in a constant position by twisting the fibre, φ was constant, and therefore, $M = K'\dot{\zeta}^2$. Now the counter-torque of the fibre produces a moment, $M' = c\Theta$, where Θ = angle of rotation of torsion head.

Since $M = M'$, $K'\dot{\zeta}^2 = c\Theta$.

Or $$\dot{\zeta}^2 = c'\Theta.$$

This is the equation relating the particle velocity to the angle of twist of the torsion head.

The first absorption band for the liquid and tube used occurred at 18,000 cycles/sec. Readings were therefore taken at about 14,000 cycles and about 23,000 cycles, corresponding to convenient frequencies above and below the absorption frequency. The results are given in Tables VIII and IX.

*Reference 4, vol. 2, p. 44.

In order to compare the experimental observations with the theoretical values, use was made of the relations,

$$\dot{\zeta}_{c_1<c} = -\alpha I_1(\alpha r) A e^{iw\left(t-\frac{Z}{c_1}\right)} \;*$$

$$\dot{\zeta}_{c_1>c} = k J_1(kr) A e^{iw\left(t-\frac{Z}{c_1}\right)} \;**$$

The average values of these velocities are,

$$\dot{\zeta}_{c_1<c} = a_1\, I_1\,(\alpha r)$$

$$\dot{\zeta}_{c_1>c} = a_2\, J_1\,(kr)\,.$$

Where a_1 and a_2 are constants. By putting a_1 and a_2 equal to unity and finding α and k, it is possible to calculate values proportional to the particle velocities.

To find α and k, we use Equations 1 and 2, in which $x = ka$, and $x' = \alpha a$.

In this experiment, water in a glass tube was employed and the physical constants are the same as were used in the calculations for the absorption frequencies.

To find αa, we use $n = 13{,}900$, and for ka, $n = 23{,}200$.

Solving Equations 1 and 2, we obtain, $x' = \alpha a = 3.42$, or $a = 3.42/3.38 = 1.01$: $x = ka = 2.44$ or $k = 2.44/3.38 = 0.722$.

Hence, $\dot{\zeta}_{c_1<c} = I_1(1.01r)$, $\dot{\zeta}_{c_1>c} = J_1(0.72r)$.

From these equations theoretical values proportional to particle velocities were calculated and are shown in Tables VIII and IX. Values of the squares of particle velocities are also shown as they are proportional to the turning moments produced on the Rayleigh disk and hence to the angles of twist (Θ) of the torsion head. In the last columns of Tables VIII and IX are values related to $[I_1(\alpha r)]^2$ and $[J_1(kr)]^2$ which have been calculated from the observed values of Θ. The first value in each group was used to determine the constant, K, relating Θ to particle velocities, while the rest of the values represent experimental observations.

TABLE VIII

Particle velocities in tube of water, *before* first absorption band

r	αr	$I_1(\alpha r)$	$\{I_1(\alpha r)\}^2$	Θ, degrees	$K\Theta$
2.6	2.63	2.83	8.01	1000	8.01
2.0	2.02	1.62	2.62	330	2.64
2.3	2.33	2.16	4.67	1320	4.67
1.6	1.62	1.11	1.23	190	0.67
1.0	1.01	0.57	0.32	—	—
0.5	0.51	0.26	0.07	—	—

The theoretical curves have been plotted in Fig. 5, and the experimental points have been marked thereon. It can be seen that the agreement is satisfactory.

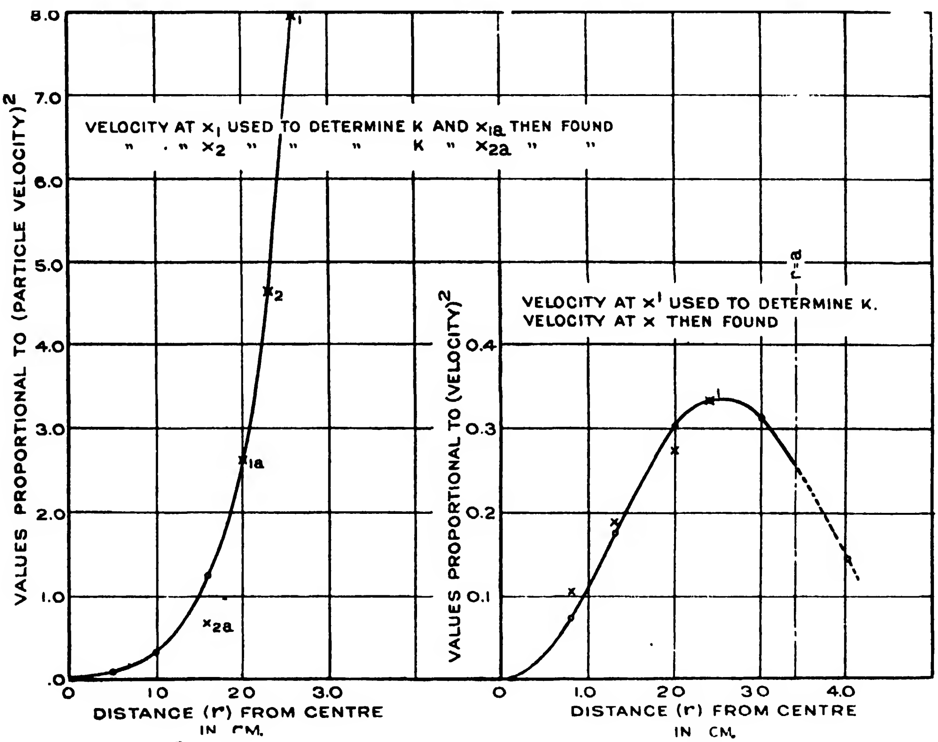
*Reference 3, p. 140, formulas 38 and 53.

**Reference 3, p. 141, formula 41.

TABLE IX

PARTICLES VELOCITIES IN TUBE OF WATER, *after* FIRST ABSORPTION BAND

r	kr	$J_1(kr)$	$\{J_1(kr)\}^2$	Θ, degrees	$K\Theta$
2.4	1.73	0.579	0.335	9×360	0.335
2.0	1.45	0.550	0.303	16×360	0.275
1.3	0.94	0.420	0.176	23×360	0.191
0.8	0.58	0.278	0.077	28×360	0.108
3.0	2.16	0.561	0.315	—	—
4.0	2.89	0.379	0.144	—	—



(*a*) (*b*)

FIG. 5. *Comparison of theoretical and experimental radial particle velocities for sound transmitted through water contained in a glass tube; (a), at a lower frequency than that of the fundamental radial vibration, (b), at a higher frequency than that of the fundamental.*

Conclusions

As a result of the foregoing experiments, it has been established that the discontinuities in the velocity-frequency curve for sound transmitted in liquids contained in cylindrical tubes are due to selective absorption of the longitudinal vibration taking place at the resonant frequencies of the radial vibration. In addition, it has been shown that the theory satisfactorily accounts for the observed facts in connection with the transmission of sound along tubes, the

phase velocities, particle velocities and the absorption frequencies having the values which the theory requires.

References

1. Boyle, R. W., Froman, D. K. and Field, G. S. Can. J. Research, 6: 102-118. 1932.
2. Boyle, R. W. and Lehman, J. F. Can. J. Research, 3: 491-509. 1930.
3. Field, G. S. Can. J. Research, 5: 131-148. 1931.
4. Raleigh, Lord. Theory of sound. Macmillan. 1926.

STUDIES OF POLYMERS AND POLYMERIZATION

IV. OBSERVATIONS ON THE POLYMERIZATION OF ISOPRENE AND 2, 3-DIMETHYLBUTADIENE-1, 3[1]

By George Stafford Whitby[2] and Robert Nelson Crozier[3]

Abstract

The polymerization of isoprene and dimethylbutadiene at temperatures ranging from 10° to 145° C. has been studied with reference to the nature of the dimeric products and the influence of the temperature and period of heating on the extent of polymerization, the ratio of oil to rubber and the molecular weight of the rubber and viscosity of its sols. There was no evidence of the formation from either diene of an open chain dimer, polymerizable to rubber, such as the "β-myrcene" of Ostromislenski. The ratio of oil:rubber from a given diene is approximately constant in different periods of polymerization at a given temperature. The ratio increases with rise of temperature. Isoprene gives far more oily by-product than dimethylbutadiene. As polymerization progresses, the molecular weight of the rubber and the viscosity of its sols rises. The higher the temperature of polymerization, the lower is the molecular weight ultimately attained. The polymerization is catalyzed by air. The viscosity of the sols of the synthetic rubbers is low and falls on keeping owing to oxidation.

Ostromislenski (27, 28) has described the preparation from isoprene, by heating, of a dimer, "β-myrcene", for which he suggested the constitution $CH_2{:}CMe.CH_2.CH_2.CH{:}CMe.CH{:}CH_2$, and which, he stated, is polymerizable to caoutchouc. If these observations can be substantiated they are clearly of first-rate significance in regard to the mechanism of the polymerization of isoprene to caoutchouc and in regard to the constitution of the latter, and the establishment of the constitution of "β-myrcene" is a matter of importance. The present work was undertaken first with the object of isolating and studying "β-myrcene" or other simple intermediate products in the polymerization of isoprene to caoutchouc. All attempts to isolate "β-myrcene" were, however, unsuccessful. The procedures described by Ostromislenski were carefully followed, but no evidence of the formation of the substance was obtained (*vide infra*). The only oily products, described later, which could be isolated when isoprene and dimethylbutadiene were polymerized by heat were cyclic dimers such as have been described by previous authors. These compounds are not further polymerizable to caoutchoucs; they are not intermediate steps in the passage from the dienes to caoutchoucs, and have no special significance in regard to the mechanism of the polymeric process by which caoutchoucs are formed.

Following the examination of the oily polymers, experiments were carried out on the influence of temperature and time of heating on the polymerization of isoprene and 2, 3-dimethylbutadiene-1, 3, with special reference to the yields of oily and rubber-like polymers respectively and with reference to the molecular weight of the rubber and the viscosity of its sols. The most important data are summarized in Table I.

[1] *Manuscript received January 30, 1932.*
Contribution from the Chemistry Department, McGill University, Montreal, based on a thesis by R. N. Crozier, April, 1928.

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TABLE I

POLYMERIZATION OF ISOPRENE AND 2, 3-DIMETHYLBUTADIENE-1, 3

Time, hr.	Isoprene				2, 3-Dimethylbutadiene-1, 3			
	Oil %	Rubber, %	Mol. wt.	Rel. visc.*	Oil, %	Rubber, %	Mol. wt.	Rel. visc.*
At 85° C.								
97.5	9.07	17.05	4589	4.62				
101	7.9	16.25			0.9	10.5	2318	3.69
154					1.5	13.8	3345	3.83
250					2.7	19.6	3524	4.55
900		35.3	5715	8.20		49.7	3483	9.21
At 145° C.								
5.25	38.2	10.03	3291	3.14				
12.5	54.7	15.56	3936	4.69	11.1	15.6	2138	2.3
At 45° C.								
2628					Trace	19.25	2106	2.76
At 10° C.								
1 year	0.005	0.01		6.13**				
At room temp.								
80 days					None	3.45	1926	1.99
4½ years	1 0	16.6	2312	13.4†				

**Relative viscosity at 30.1° C. of sol containing approx. 0.68 gm./20 cc. benzene.*
***Relative viscosity at 30.1° C. of sol containing approx. 0.492 gm./20 cc. benzene.*
†First fraction (see page 210).

The results at 145° C. are in good accord with those obtained by Lebedev (20, p. 1313) at 150° C. At this temperature he found the total polymerizate from isoprene to be 53% after five hours and 79% after 15 hr., while from dimethylbutadiene the total polymerizate after 15.5 hr. was 39.7%. He found that when the polymerization of isoprene at 150° C. was complete, the product contained only 10% of rubber.

As an examination of Table I shows, the rate of polymerization of isoprene when heated is greater than that of dimethylbutadiene. This is perhaps somewhat unexpected, as the authors have observed, in accord with most previous workers, that at room temperature dimethylbutadiene undergoes polymerization far more readily than isoprene. Samples of the former were generally observed to undergo complete polymerization to typical, white, cauliflower masses when kept for a year or two (Kondakov (12), who first observed the spontaneous polymerization of dimethylbutadiene, found it to be complete in one year), whereas a sample of isoprene kept for 4½ years showed no separation of solid polymer and only about 16% polymerization to caoutchouc.

Even when considerably diluted, samples of dimethylbutadiene were observed by Macallum and Whitby (23) to undergo on keeping conversion to the solid polymer after two or three years. Thus, *e.g.*, a fraction prepared from pinacone, boiling at 90 to 100° C. and containing a large proportion of pinacoline, showed on keeping considerable separation of polymer.

Heat polymerization leads to the formation of a much larger proportion of oily by-products in the case of isoprene than in that of dimethylbutadiene. Increase in the temperature applied raises the proportion of oil to caoutchouc in both cases. In the experiments at 145° C. the oil formed from isoprene is more than three times the amount of caoutchouc formed. This makes it clear that the temperatures specified for the polymerization of isoprene to rubber in certain patents (*e.g.*, 4) are too high.

Some uncertainty attaches to the figures given for the molecular weight of the polymeric products, owing to the fact that the results of cryoscopic determinations were affected by the concentration of the solutions used. The results may, however, be considered as approximately correct, and, regarded broadly, show that, on heating either of the dienes at a given temperature, the molecular weight of the rubber produced increases as the percentage of the diene which has undergone polymerization rises. Further, the higher the temperature applied to produce polymerization, the lower is the molecular magnitude of the rubber produced after similar amounts have been formed. And, again, as polymerization at a given temperature progresses, the viscosity of sols of the product rises. These generalizations are all in accord with those established by Whitby and Katz (37, 38) in a study of the polymerization of indene, namely, that, as polymerization by heat progresses at a given temperature, the state of polymerization rises, and the higher the temperature applied, the lower is the state of polymerization which is attainable in the ultimate product.

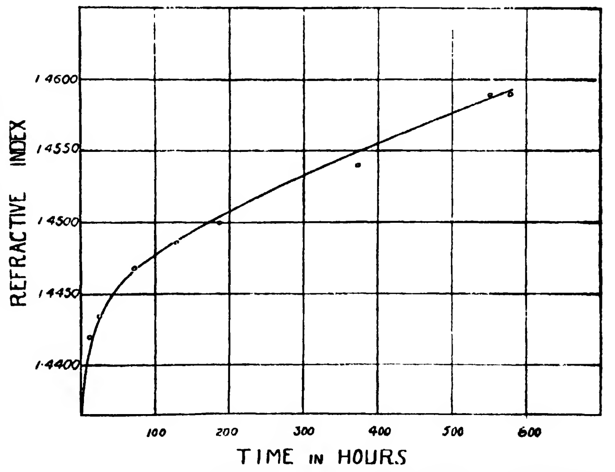

FIG. 1. *Change of refractive index of dimethylbutadiene with heating at 85°C.*

Since the polymerization of dimethylbutadiene at 85°C. is accompanied by the formation of only small amounts of oily, dimeric by-products, it was possible to follow the course of the polymerization by following the change of the refractive index. The results obtained in this connection are illustrated by the curve in Fig. 1. The refractive index changed rapidly at first, and then the rate of change slowed up considerably. This is in accord with the prior experiments (the results of which are given in Table I), in which

the polymerization products were isolated and weighed; polymerization proceeds rapidly up to a certain point and then slows down. During the first 24 hr. heating at 85° C. the refractive index of dimethylbutadiene rose from 1.4367 to 1.4435, whereas after heating for 15 days and 12 hr. it rose only to 1.4540.

The rubber obtained by the polymerization of dimethylbutadiene at 85° C. dispersed in a few hours when placed in benzene, whereas that obtained by its polymerization at room temperature, which is presumably of much higher molecular weight, only swelled in benzene and had not dispersed after two months' standing.

By fractional precipitation it was found possible to separate dimethylbutadiene caoutchouc into fractions which gave sols possessing, at the same concentration, different viscosities. This shows that the polymeric products consist of a mixture of polymers representing different degrees of polymerization. A similar heterogeneity has previously been shown in the case of polymerizates from indene (37, 38), vinyl acetate (40) and styrene (31, 39).

Measurements made of the iodine absorption of dimethylbutadiene caoutchouc point to the presence, as in natural caoutchouc, of one double bond for each unit of the diene which has gone to form the polymer. This is in agreement with the molecular refraction as determined by Macallum and Whitby (22).

Sols of the caoutchoucs obtained by the heat polymerization of both isoprene and dimethylbutadiene were observed to suffer a marked decrease in viscosity on keeping. For instance the time of flow of a sol of dimethylbutadiene caoutchouc which was originally 155.4 sec. had fallen after only 25 days in the dark to 81.6 sec., and that of a sol of isoprene caoutchouc from a time of flow of 221.2 sec. 7 days after its preparation to 125.2 sec. after 46 days. That the fall in viscosity is due to the gradual oxidation of the synthetic rubber is made apparent by the further observation that the addition of an antioxidant, dimethylaniline, prevented a fall in the viscosity on keeping. Fig. 2 shows the fall in viscosity of sols of methyl caoutchouc with time. The fall resulted from a permanent change in the caoutchouc, since on recovering the polymer from a sol which had been kept it was found to have been

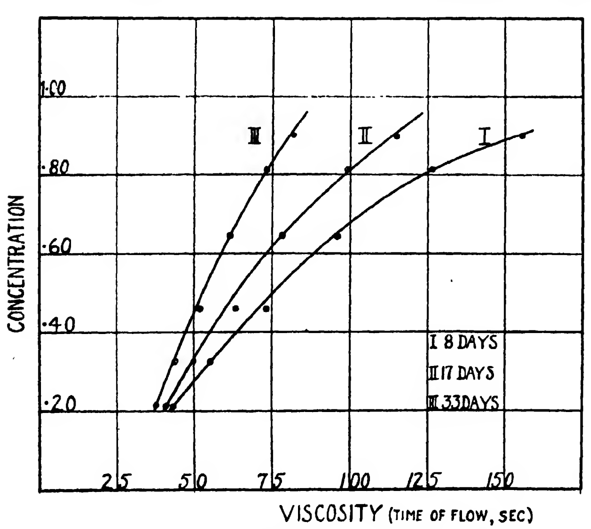

Fig. 2. *Fall in viscosity of sols of dimethylbutadiene caoutchouc on keeping. Time of flow measured after keeping sols for 8, 17 and 33 days.*

converted into a sticky product different from the originally tough, elastic rubber.

Various inorganic halides were found to bring about the rapid catalytic polymerization of isoprene and dimethylbutadiene in the cold. The most vigorous catalyst observed appeared to be antimony pentachloride. Other powerful catalysts were stannic chloride, antimony trichloride and aluminium chloride. Less effective were ferric chloride and bromide, thorium bromide, boron trichloride and tribromide and dichloracetic acid. These yielded a small quantity of polymer after standing two days. The polymers produced by the various halides were in all cases white or pale-colored substances, inelastic and quite unlike rubber. Apparently they are isomers of rubber. It is interesting to note that the halides converted natural rubber into similar products. On adding them to a solution of rubber in benzene highly colored complex products were formed first, and these, when alcohol was added, yielded white or pale-yellow powders, mostly soluble in benzene to only a slight extent. A similar observation in the case of the action of stannic chloride on rubber has been made by Bruson, Sebrell and Calvert (6).

Dimers

By heating isoprene at 280-290° C. for six hours, G. Bouchardat (5) obtained among other products a terpenic fraction which boiled chiefly at 176-181° C., and had d^{0} 0.866, d^{21} 0.853. It was designated terpilene. Tilden (32) heated isoprene at 250-280° C. for 12 hr. and found on distillation that about half the material came over between 170 and 180° C. Wallach (34) identified dipentene in oils such as those just mentioned. He heated isoprene for some hours at 250-270° C. and obtained, together with high boiling polymerization products, a hydrocarbon, boiling at 180° C., which yielded a tetrabromide identical with dipentene tetrabromide. Wallach (34) also identified dipentene in the oil obtained by the destructive distillation of rubber, and it came to be generally considered that dipentene constitutes the major part of the terpene fraction in the oil obtained by the dry distillation of rubber. Harries (8) however, states that dipentene constitutes at most one-third of the "dipentene fraction" boiling at 150-200° C. obtained from rubber.

Harries further states that on heating isoprene at 300° C. he found only a little dipentene to be formed, the main product being another terpene boiling at 64-66° C./12 mm.

Lebedev (18, 19, 21) subjected isoprene to a lower temperature than the temperatures mentioned above, namely, 150° C. He obtained besides dipentene another dimer which he considered to be 1,3-dimethyl-3-ethenyl-6-cyclohexene. The properties of the two fractions he obtained were as shown in Table II.

In the experiments recorded in the present paper Lebedev's dimer was not encountered. Harries (9) in a later paper than that mentioned above reports on the terpene fraction of freshly polymerized isoprene, without however specifying exactly the conditions under which polymerization was carried out, although apparently they involved heating isoprene with acetic acid at a tem-

TABLE II

PROPERTIES OF FRACTIONS OBTAINED BY LEBEDEV ON HEATING ISOPRENE TO 150° C.

Fraction	B.p. at 760 mm.	B.p. at 9 mm.	d_0^{20}	n_D^{20}
No. 1 (Lebedev's dimer)	160-161° C.	44°	0.8331	1.46581
No. 2 (Dipentene)	174-175° C.	58°	0.8454	1.47428

perature of about 100° C. The main quantity he found to boil at 63-65° C./14 mm. and to show d_{18}^{18} 0.8451, n_D^{18} 1.4708. It gave only a little dipentene tetrabromide. Harries considered that it probably consisted for the greater part of an open-chain dimer of isoprene of the same formula as that later assigned to "β-myrcene" by Ostromislenski (*infra*). The product was not investigated further. Aschan also has reported on a dimeric product from isoprene, which he regards as quite distinct from dipentene, but which he classifies as a cyclic compound of the sylvestrene series, and to which he assigns the name diprene. After being allowed to stand at ordinary temperature for 10 years, he found, that 250 gm. of isoprene yielded (2) 7 gm. of dimeric material, the major fraction of which had the properties recorded below and yielded a dihydrochloride which melted at 51.5-52° C. and depressed the melting point of dipentene dihydrochloride when mixed with the latter (m.p. of dipentene hydrochloride: 48-50° C.). Aschan (1, 3) also examined somewhat larger quantities of dimeric material obtained by the polymerization of isoprene "at temperatures below 100° C." (it is not stated whether a catalyst was used in the polymerization), and concluded that this too consisted mainly of diprene. The physical properties of the main fraction obtained by distillation under ordinary and under reduced pressure are shown below (*b* and *c*). A sample of "diprene" isolated in the present investigation from isoprene which had been allowed to stand at room temperature for 4½ years was proved to contain dipentene by the preparation from it of dipentene tetrabromide.

TABLE III

PROPERTIES OF ASCHAN'S DIPRENE

Polymerization conditions	Boiling point, °C.	d_4^{20}	n_D
(a) By cold polymerization of isoprene	171-172/769 mm.	0.8535	
(b) By polymerization of isoprene below 100°	171.5-173/752 mm.	0.8481	1.46960*(23.4° C.)
(c) By polymerization of isoprene below 100°	68.5-69/16 mm.	0.8476	1.46946(23.2° C.)

**The figure given in the reference, viz. 1.49660, is apparently due to a clerical error.*

With the possible exception of the dimeric products mentioned by Harries (9) all the dimers just described as being obtained from isoprene are clearly cyclic compounds. The observed refractive index for both Lebedev's and Aschan's

products agree reasonably well with the calculated value for $C_{10}H_{16}\overline{|}_{2}$. An entirely different dimer, *viz.*, "β-myrcene", has however been described by Ostromislenski (27, 28). It is stated that this hydrocarbon is formed when isoprene, either alone or in the presence of a catalyst, is allowed to stand at ordinary temperature or is heated to not above 150° C. (27). It is stated to be the chief product when isoprene is heated to 80-90° C. for 3-5 days (28). It is also stated to be an open chain compound containing three double bonds of which two form a conjugated system; to be polymerizable quantitatively to caoutchouc; to yield, like other conjugated hydrocarbons, an amorphous solid with an aqueous solution of sulphur dioxide. It is distinguished from the dimers of isoprene described by all other authors in possessing a much higher refractive index than they. The present authors find, it may be mentioned here, that the value recorded by Ostromislenski is too high even for the assumed constitution.

"β-myrcene"

B.p. 63.5°C./20 mm. n_D^{26}, 1.53681. d_4^{20}, 0.8472. MR_D: Found, 50.01; calcd., 47.8 (for $C_{10}H_{16}\overline{|}_{3}$ +0.98 for exaltation).

As early as 1900-1902 Kondakov (14) had suggested "that a transient stage in the polymerization of these hydrocarbon conjugated dienes consists of dimeric, open chain isoprenes, containing several double bonds in the molecule." But prior to Ostromislenski's work on β-myrcene there was no clear record of the isolation of such an open chain dimer. In the present investigation attention was particularly directed to β-myrcene when studying the oily dimers from isoprene. The conditions under which the polymerization of isoprene were brought about were all conditions which Ostromislenski has indicated as suitable for the preparation of β-myrcene, but in no case was any evidence found of the presence of this material, which should be so easily recognizable on account of its high refractive index. None of the products underwent polymerization when treated with sodium and barium peroxide as directed by Ostromislenski, and none reacted with sulphur dioxide solution except apparently in so far as some of the fractions were contaminated with isoprene. The iodine absorption of one of the products was determined and found to correspond to the presence of two double bonds only. Further, the oil obtained in one experiment was subjected to bromination and proved to contain a considerable proportion of dipentene.

The temperatures at which isoprene was polymerized were (a) room temperature, (b) 85-92° C., (c) 145° C. Polymerization was also carried out at 90° C. in the presence of benzoyl peroxide. The physical properties of the main fractions are shown in Table IV.

A definitive investigation of the dimeric polymerizate from isoprene probably demands the careful fractionation and study of a much larger quantity of material than has been employed by any workers hitherto. In the opinion of the present authors the material is preponderantly dipentene. The fact that on bromination it yields a considerable amount of oily bromide in addition to

TABLE IV

DIMERIC FRACTIONS FROM ISOPRENE

Polymerization conditions	Boiling point, °C.	n_D	d_4^{25}
Expt. A. 5 days at 92° C.			
Fraction 2	172-174 (760 mm.)	1.4750 (16.5° C.)	
Fraction 3	174-176 (750 mm.)	1.4753 (16.5° C.)	
Expt. B. 98 hr. at 85° C.			
Fraction 3	171-174 (760 mm.)	1.4705 to 1.4710 (26° C.)	
Fraction 4	174-175 (760 mm.)	1.4723 to 1.4726 (26° C.)	
Expt. E. 10 days at 90° C. with 5% benzoyl peroxide			
Fraction 1	60-61.5 (10.5-11 mm.)	1.4710 to 1.4713 (25° C.)	0.8320
Fraction 2	61.5 (10.5-11 mm.)	1.4727	0.8380
Expt. F. 4½ years at room temperature		1.47120 (23.4° C.)	

solid dipentene tetrabromide can be explained by the presence of a relatively small amount of material other than dipentene, as the bromination of even a stock sample of redistilled dipentene from another source gave a fair amount of oil along with the solid. The formation of dipentene from isoprene over a wide temperature range has now been established. Wallach (34) shows that it is formed at 250-270° C.; Lebedev (18-21) that it is formed at 150° C.; and the present authors that it is formed at 92° C. and at room temperature. Another point to be noted is that, in the isolation of the dimeric polymerizate of isoprene, even after the apparently thorough removal of unchanged isoprene by distillation on the water bath at ordinary pressure, the dimeric material was observed to be contaminated with isoprene (see Expt. B below). The presence of such contamination in the products of earlier investigators may possibly account for some of the deviations in physical properties recorded for the products.

As in the case of isoprene, an examination of the dimeric polymerizate from dimethylbutadiene failed to indicate the presence of an open-chain compound, the products obtained being cyclic ones similar to those described by previous workers.

Experimental

Preparation and Purification of Isoprene

Crude isoprene was prepared from dipentene* by means of a modified "isoprene lamp"†. The essential features of the apparatus are shown in Figs. 3 and 4. In Fig. 3, *A* is a three-litre Pyrex flask containing the heating element *B* which serves to crack the dipentene vapor. *C* is a condenser (with inner tube of Pyrex glass) through which water at 45-50° C. is circulated. *D* is a spiral condenser (with more coils than shown in the diagram) through the jacket of which cold water is circulated. *E* is a 300-cc. flask cooled to −10° C. by means of an ice-salt mixture in the bath *F*. *G* is placed in a Dewar

**Kindly supplied by the Hercules Powder Company.*

†Cf. Reference No. 10.

flask, *H*, filled with ether cooled by solid carbon dioxide. It serves to collect any isoprene which escapes from *E*.

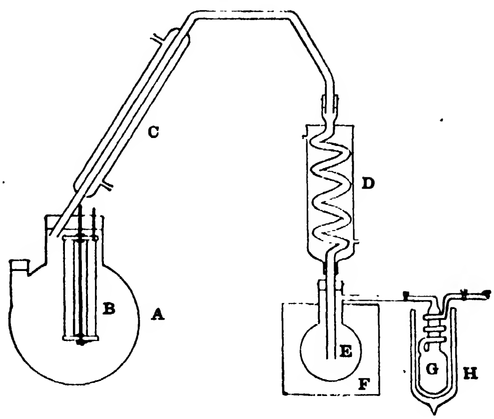

FIG. 3 *Modified "isoprene lamp."*

Fig. 4 shows diagrammatically the heating element, *B*, the upper diagram being a plan. The element consisted of six feet of No. 28 gauge platinum wire, *F*, threaded through holes in two perforated disks of asbestos slate, *B*, 4.75 in. apart, and forming eleven vertical strands. The holes in the plates for the passage of the wire were made as large as the strength of the plates would permit, in order to allow of the free passage of vapors. The plates, 0.125 in. in thickness and 1.5 in. in diameter, were attached to a copper wire, *C*, 0.125 in. in diameter, by means of nuts soldered to the rod on either surface of each plate. This rod acted as one terminal. The other terminal, *E*, was attached to the end of the platinum wire which had been brought over the edge of the upper plate. It may be mentioned that a nichrome element could not be used in place of platinum, as after a few minutes' running it became heavily coated with carbon.

In addition to the cracking apparatus just described, an apparatus for cracking under reduced pressure was built on lines similar to those described by Staudinger and Muntwyler (25). It was, however, found to be considerably more inconvenient and troublesome than the former apparatus.

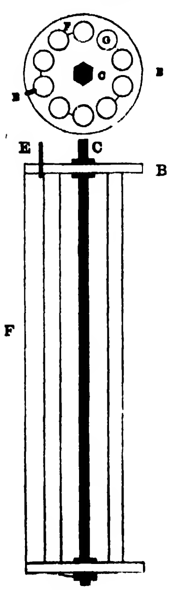

FIG. 4. *Cracking element for isoprene lamp.*

In operating the "lamp" described the charge of dipentene was 450 cc. only, as with this charge the apparatus worked most efficiently. After the charge had been brought to the boiling point and the air displaced, the heating coil was brought to, and maintained at, a dull red heat by 7.5 to 8.5 amp., 110 volts d.c., while the dipentene was kept boiling fairly rapidly. Owing to the gradual deposition of carbon on the platinum, the efficiency fell as the run proceeded. Hence each morning before starting a run the platinum was cleaned by heating the coil to a bright red heat. Further, as a run proceeded accumulation of resins in the liquid left in the cracking flask led to a falling-off in the rate of isoprene formation. Hence before each run the flask was cleaned out. In a typical run a charge of 450 cc. of dipentene gave in seven hours a distillate of 270 cc. and left a residue of 160 cc. The distillate gave on redistillation 190 cc. of an isoprene fraction (b.p. 27.5 to 37° C.), the balance being unchanged dipentene.

Four litres of crude isoprene prepared by the lamp was dried over calcium chloride and fractionated several times. The method of purification at first employed was one described by Ostromislenski (26). Isoprene (1500 cc.) boiling at 33-36° C. was allowed to stand at room temperature over 16 gm. of sodium wire and 135 gm. of barium peroxide for at least two days; it was then refluxed for one hour and fractionated. This gave 1300 cc. of isoprene boiling at 33-35° C. As it appeared in the first experiment (Experiment A) on polymerization, that the above method does not yield isoprene of very high purity, the material used in all but the first experiment was subjected to further purification involving conversion to the tetrabromide*.

Following the procedure of Gustavson (7) 100 cc. of isoprene, dissolved in 100 cc. of chloroform, was introduced into a two-litre flask fitted with a reflux condenser, dropping funnel and mechanical stirrer. The flask was cooled in a bath to −10° C. and a previously cooled solution of 320 gm. of bromine in 500 cc. of chloroform was added drop by drop. It was found after five or six experiments that these concentrations were the most convenient. In more concentrated solutions a certain amount of hydrobromic acid was evolved. The solution of bromides thus obtained was washed with aqueous soda solution until colorless and the chloroform layer was dried over anhydrous potassium carbonate. The chloroform was distilled off and the concentrate, generally brown in color, distilled *in vacuo*. The three main fractions were as follows:— I. 60-70° C. at 12 mm. This, containing amylene bromides, was discarded. II. 88-94° C. Isoprene dibromide. III. 155-160° C. Isoprene tetrabromide. The yield of the second and third fractions was about 75-80%. After three or four distillations *in vacuo*, colorless, or almost colorless, products were obtained. As they turned dark on standing, they were used at once. Only a small proportion of the dibromide was obtained.

The regeneration of the isoprene from the tetrabromide was carried out as follows:— 320 gm. of isoprene tetrabromide was added slowly by means of a dropping funnel to 450-500 cc. of alcohol in which was suspended 262 gm. of zinc dust, the latter being added in three portions, and the reaction mixture being kept in constant agitation by an electrically driven stirrer through one neck of the flask (three-necked type). To this flask was attached a reflux condenser kept at a temperature of 45°-50° C. The rest of the apparatus for collecting the isoprene was similar to that in Fig. 3. Traces of alcohol carried over with the isoprene were removed by washing. The yields by this method were 85 to 90% of the theoretical. If stirring was not applied, they were much poorer. The same procedure was applied for the regeneration of the isoprene from the dibromide fraction, the yields being 75-85%.

Finally the regenerated isoprene was dried over calcium chloride and distilled. The distillate was then allowed to stand for several days over sodium wire and redistilled. The boiling point of the pure product was 33.4 to 33.9° C. at 744 mm.

**Cf. References 15 and 24.*

Preparation of 2, 3-Dimethylbutadiene-1, 3

Pinacone hydrate was prepared from carefully dried and redistilled acetone by reduction with amalgamated aluminium as follows. To 200 gm. of 20-mesh aluminium in a perfectly dry three-litre flask a solution of 30 gm. of mercuric chloride in 300 cc. of acetone was added rapidly with agitation. The mixture was left undisturbed for 15-20 min., and then 10 gm. of finely divided zinc chloride and 100 cc. of acetone was added. The mixture was allowed to stand 1½-2 hr., with frequent vigorous agitation, to prevent caking of the aluminium. Then 500 cc. acetone and a solution of 30 gm. of mercuric chloride in 600 cc. of acetone was added dropwise over a period of four hours at such a rate as to keep the liquid boiling, 100 cc. of one and 100 cc. of the other reagent being added alternately. The flask, covered with a cloth, was allowed to stand overnight. Generally it was found refluxing slowly in the morning. In all the reaction took 35-40 hr. Moisture was excluded. Stirring was not found to be necessary so long as the aluminium was kept in a loose condition. Finally the flask was heated on the water bath for two hours. Then 1200 cc. of water was added gradually to decompose the complex, the mixture was boiled for two hours, allowed to cool to 40° C., and filtered. The mass was extracted with four 1000-cc. portions of acetone. The filtrate and washings were concentrated to about 2000 cc. and the supernatant oil removed. The aqueous liquid on standing overnight in an ice box deposited crystals of pinacone hydrate. The oil, which consisted of mesityl oxide, phorone, etc., deposited crystals of pinacone. The total yield of pinacone hydrate was about 350 gm. The product was recrystallized twice by dissolving it in an equal weight of hot water, filtering and cooling in ice. Water of crystallization was removed from the pinacone hydrate by distillation, practically all the water being given up under 135° C. Pinacone carried over to the extent of 10-15% was recovered. A long air condenser and well-cooled receiver were used. Yield: 85-90%. The use of potassium hydroxide and potassium carbonate as dehydrating agents was not as convenient as the method described.

For conversion of pinacone into the diene the following gave the best yields of the methods tried. Pinacone (500 gm.) was distilled with 2 gm. 48% hydrobromic acid (16), half of the pinacone being added through a separating funnel. The reaction proceeded very slowly, but after 24 hr. most of the pinacone had reacted. The hydrocarbon layer of the distillate gave after one fractionation 55% of the theoretical yield of dimethylbutadiene boiling from 68 to 72° C. By repeated fractionation of the higher boiling fractions a further quantity of diene was recovered, the total yield from 3000 gm. of anhydrous pinacone being 1600 gm. of dimethylbutadiene (75%).

Conversion of pinacone to the diene by means of potash alum, by means of sulphanilic acid—methods used by Macallum and Whitby (23)—or by means of potassium bisulphate did not give as good yields as the procedure just described.

Impurities in the diene, mainly pinacoline, were removed by treating the carefully dried and fractionated product with sodium. After several such

treatments, the final product had—b.p. 69.5-70.5° C.; n_D^{22}, 1.4370 (one sample); n_D^{24}, 1.4367 (another sample). Kondakov gives n_D^{20} 1.4375; and Macallum and Whitby (22), n_D^{20} 1.4376.

Polymerization of Isoprene

Examination of the Oily By-products

Experiment A. Following a procedure of Ostromislenski (28) for the preparation of "β-myrcene", 200 gm. of isoprene was heated in an autoclave for five days at a temperature ranging from 85 to 95° C., but for the most part at 92° C. Unchanged isoprene (56% of the total) was removed from the resulting colorless, syrupy liquid by distillation from a water bath. The residue was then distilled with steam and gave 65 cc. (27% of the isoprene used) of a clear, colorless oil and 30 gm. (15%) of a rubber-like residue.

The oil, which had a peculiar odor unlike that of a stock sample of dipentene, was dried in ether over anhydrous sodium sulphate, and then distilled under 10.5 mm. It distilled over between 53.5 and 56.5° C., leaving a small residue of very viscous, colored oil. The residue when treated with alcohol gave a fine crystalline precipitate, the nature of which was not investigated.

Examination of the distillate gave the following results:— mol. wt.. (cryoscopically, in benzene), 139.9 (calcd. for $C_{10}H_{16}$: 136.2); $n_D^{14.5}$, 1.4769. (A sample of dipentene gave $n_D^{14.5}$, 1.4765.) It gave only a trace of amorphous,

TABLE V

Iodine absorption, by Hanus' solution, of oil obtained in Experiment A

Period of absorption (hr.)	5	5.5	7.5	24
No. of double bonds	1.8	1.8	1.9	2.1

white precipitate when treated, even for several days, with a concentrated aqueous solution of sulphur dioxide. When heated according to Ostromislenski's instructions (28) with sodium and barium peroxide in a glass tube for three days at 100° C., it gave no evidence of the occurrence of polymerization.

Distillation under atmospheric pressure yielded the following fractions. There was no sign of polymerization during this distillation.

Fraction 1, up to 172° C. (corr.). Fraction 2, 172-174° C.; n_D/16.5° C., 1.4750. Fraction 3, 174-176° C.; n_D/16.5° C., 1.4750. Fraction 4, 176° C.-end.

Fractions 2 and 3, which comprised most of the oil, were shown by bromination to contain a considerable proportion of dipentene. On distilling a sample of dipentene most of it came over between 174 and 176° C. (b.p. of pure dipentene is given as 175-6° C.). As Fraction 2 above was equal in volume to Fraction 3, it appears that the oil from isoprene contains one or more other cyclic dimers in addition to dipentene. The bromination results are also in agreement with this assumption.

Samples of Fractions 2 and 3 and also of dipentene were diluted with four parts of absolute alcohol and four parts of absolute ether and saturated in the

cold with bromine. They were allowed to stand in an ice box for two weeks, and the solutions were then evaporated. The residue consisted in each case of crystals mixed with a very viscid oil, but from dipentene the crystals formed a greater proportion of the whole than from the fractions of the other oil. After several recrystallizations from ether each of the batches of crystals melted at 125° C.

Experiment B. Since the isoprene used in Experiment A had been purified only by means of sodium and barium peroxide, and since an iron autoclave had been used, the experiment was repeated using sealed glass test tubes and highly purified isoprene (from the tetrabromide). Pure isoprene (300 cc.) was heated in six sealed glass tubes at 85° C. for 98 hr., all the details given by Ostromislenski in one place* for the preparation of β-myrcene thus being very exactly duplicated. Unchanged isoprene was removed by distillation at atmospheric pressure and the oil was then separated from the rubber-like high polymer by distilling, until no further loss in weight occurred, at a pressure of 9 mm. which was later reduced to 1 to 2 mm. The distillate was collected in a flask cooled in solid carbon dioxide and ether. The oil, amounting to 19.5 gm., was dried over calcium chloride and again distilled under a pressure of 7 mm. Only a very slight residue remained. Re-fractionation of the oil gave the following results at 7 mm. Hg. Fraction 1: 7.3 gm.; b.p., up to 53°/7 mm., mostly at 52-53° C.; n_D^{25} 1.4540. Fraction 2: 11.4 gm.; b.p., 53-54° C./7 mm.; n_D^{25}, 1.4713. Fraction 3: 0.75 gm., residue. The low refractive index of Fraction 1 was shown to be due to the presence of some isoprene.

These fractions were then distilled at atmospheric pressure with the results shown in Table VI.

TABLE VI

PROPERTIES OF FRACTIONS OBTAINED FROM EXPERIMENT B

Fraction	Boiling point °C.	Refractive index at 26° C.	Behavior with an aqueous solution of SO_2
1. Isoprene present	Up to 128		Gave a heavy, white precipitate.
2.	128-171 (mainly 169-171°)	1.4667 to 1.4670	Slight precipitate.
3.	171-174	1.4705 to 1.4710	Very slight precipitate.
4.	174-175	1.4723 to 1.4726	No precipitate.
5. Residue (slightly brown viscid oil)		1.4827 to 1.4820	

The residue was distilled under reduced pressure and a few drops of a clear, colorless oil were obtained; n_D^{25}, 1.4730.

None of the fractions obtained (discarding No. 1) showed any tendency to polymerize when treated with sodium and barium peroxide for three days at 100° C.

Clearly the oil obtained in this experiment was substantially the same as that obtained in Experiment A.

*Cf. Reference 30, p. 94.

Experiments C and D. In these experiments a higher temperature was applied, isoprene being heated for 5.25 and 12.5 hr. respectively at 145° C. The oil proved to be essentially similar to that obtained in the two preceding experiments, as was shown by the boiling point and refractive index of the fractions into which it was separated.

Experiment E. In a second procedure given by Ostromislenski for the preparation of β-myrcene (27) pure isoprene is heated with 5% of benzoyl peroxide for 10 days at 90° C. Using the same procedure, 50 cc. of pure isoprene and 1.7 gm. of benzoyl peroxide were introduced into each of three Pyrex tubes, sealed and heated as above-mentioned. As in previous experiments, most of the isoprene remained unchanged; after its removal the residue was steam distilled, the oil separated, dried over calcium chloride and distilled under reduced pressure. At 10.5 mm. of mercury the boiling point rose rapidly to 61.5° C. and remained there until the close of the distillation. Two fractions were obtained. Fraction I.: b.p., up to 61.5° C. (mostly at 60-61.5°) at 10.5-11 mm.; n_D^{25}, 1.4710 to 1.4713; D_4^{25}, 0.832. Fraction II.: b.p., 61.5° at 10.5-11 mm.; n_D^{25}, 1.4727; D_4^{25}, 0.838.

The oil fractions behaved in a similar way to those obtained in preceding experiments.

No substance could be isolated in these experiments in any way comparable to the behavior of the so-called "β-myrcene".

Experiment F. About 50 cc. of isoprene was allowed to stand for 4½ years at room temperature in the dark in a bottle of 100-cc. capacity, the space above the liquid being filled with air. At the end of this period no separation of polymer had taken place, but the liquid, although not highly viscous, was noticeably more viscous than the original isoprene. The major portion was distilled at ordinary pressure from a water bath in order to remove unchanged isoprene; the dimeric oil was then removed by distillation at 17 mm., and the residue of crude rubber was weighed: 31.2 gm. gave 0.31 gm. oil (1%) and 5.2 gm. (16.6%) rubber.

The oil, colorless, possessed a peculiar and somewhat ethereal odor. n_D, 1.47125 at 23.2° C., 1.4710 at 23.6° C. It was brominated in solution in absolute alcohol and ether and the solution was allowed to stand in a refrigerator for 24 hr. The crystals which had separated were isolated and after five recrystallizations from ether containing a little alcohol gave a colorless product melting at 123.5-124.5° C. A sample of dipentene tetrabromide prepared in the same way from redistilled dipentene melted at 124.5-125° C. A mixed melting point determination gave 123-124° C.

The crude rubber (5.2 gm.) was dissolved in 40 cc. of benzene and poured dropwise into 200 cc. of absolute alcohol. A white rubbery clot formed. It was removed and dried in a vacuum until constant in weight (1.5875 gm.). When dry it was clear, colorless, elastic but not strong. Data for the molecular weight of this fraction and for the viscosity of its sols are given in Table VII. The mother liquor from the above precipitate was evaporated to 25 cc. and a further quantity (75 cc.) of absolute alcohol was added. This precipitated a

second fraction of rubber (2.3 gm.), which, unlike the first fraction, was yellowish and somewhat sticky. Evaporation of the mother liquor gave a third fraction, also somewhat sticky and noticeably darker in color.

In addition to the isolation of rubber from the 4½-year old isoprene by distillation, rubber was isolated from a portion by precipitation. The old isoprene (3.6578 gm.), treated with 50 cc. of methyl alcohol, yielded a clot of colorless rubber weighing 0.2218 gm. The mother liquor was then evaporated, the residue was taken up in 5 cc. benzene, and more precipitant was added. This gave a further quantity of 0.1496 gm. rubber. The total rubber recovered was thus 10.2%. The mother liquor contained a further fraction which presumably was in such a low state of polymerization that it was difficult to precipitate it.

Effect of Temperature and Time on the Polymerization of Isoprene

After heating pure isoprene at the temperatures and for the periods specified, unchanged monomer was removed by distillation at ordinary pressure; then oily polymeric products were removed by distillation under reduced pressure until the distillation flask suffered no further loss in weight, and the yield of rubber was obtained by weighing the tared flask. The oil was collected in a receiver cooled with solid carbon dioxide and ether. The yield of oil was determined by evaporating the distillate under reduced pressure until no more diene came off. As the speed of polymerization is influenced by the volume of air in contact with the diene, a constant ratio of diene to air was employed in all but one of the experiments at 85° and 145° C. The tubes were of 165-cc. capacity and each contained 50 cc. of isoprene except in the experiment in which a heating period of 101 hr. was applied. In the latter the tubes had a capacity of 90 cc. and contained 30 cc. of diene. The results are given in Table I.

Molecular Weight of Rubber Polymers and Viscosity of Their Sols

The samples of synthetic rubber obtained in the above polymerization experiments were purified by dissolving them in pure anhydrous benzene and precipitating them with absolute alcohol, this procedure being repeated at least twice. After the greater part of the attached liquid had been expressed from the precipitated rubber, the latter was dried for 4-5 days in a desiccator evacuated by means of a Hyvac pump. All measurements were made immediately the samples were dry, as it was observed that even when they were kept in a vacuum of 9-10 mm. oxidation occurred rather rapidly.

Molecular weights were determined cryoscopically in benzene. In preparing the solutions, bottles containing the rubber and benzene were kept in the dark for four days with occasional shaking. Results obtained at different concentrations are shown in Table VII. Average values have been recorded in Table I.

A uniform procedure was followed in the preparation of sols of the samples for viscosity measurements, the samples being treated with 20 cc. of purified benzene in a 50-cc. bottle and kept for eight days in a dark cupboard, with shaking once a day. Measurements were made with an Ostwald viscosimeter at 30.1° ± 0.02° C.

TABLE VII
ISOPRENE CAOUTCHOUC

Sample, time and temp. of polymerization	Viscosity: Conc., gm./20 cc. benzene	Viscosity: Relative viscosity, benzene = 1	Molecular weight: Conc., gm./20 cc. benzene	Molecular weight: Mol. wt.
(a) 97.5 hr. at 85° C.	0.1290	1.54	0.2627	3348
	0.2025	1.81	0.6808	4870
	0.4710	3.10	0.6810	4893
	0.4997	3.24	0.6862	5158
	0.6810	4.62	0.8292	4680
	0.6862	4.66		(Mean:
	0.7285	4.79		4589)
	0.8292	5.92		
	0.9316	6.44		
(b) 900 hr. at 85° C.	0.1077	2.01		
	0.2946	3.92		
	0.6830	8.20	0.6830	5562
	0.7004	9.43	0.7004	5867
	0.8098	8.84*		(Mean:
	0.9223	11.85		5715)
(c) 5.25 hr. at 145° C.	0.0715	1.14		
	0.2406	1.57	0.2406	2713
	0.5078	2.36		
	0.6681	3.09		
	0.6935	3.14	0.6935	3630
	0.7380	3.20	0.7380	3360
	0.9325	4.14	0.9325	3460
				(Mean:
				3291)
(d) 12.5 hr. at 145° C.	0.2317	1.97	0.2317	2613
	0.4440	3.03	0.4440	3617
	0.6947	4.65		
	0.6982	4.69	0.6982	4761
	0.8268	5.78	0.8268	4753
				(Mean:
				3936)
(e) 1 year at 10° C. ±	0.0512	1.24		
	0.1172	1.60		
	0.2912	2.57		
	0.4920	6.13		
(f) 4½ years at room temp.	0.2907	5.0	0.4915	2308
	0.4915	7.9	0.6797	2315
	0.6797	13.4		(Mean:
				2312)

*Measurement after 10 days.

Cold, Catalytic Polymerization of Isoprene and Dimethylbutadiene

To quantities of 0.5 cc. of isoprene a number of halides were added and the following observations made.

(*a*) *Stannic chloride* (2 drops). Polymerization complete in three hours yielding a clear, soft, amber colored, non-elastic mass, which when warmed showed elastic properties if squeezed, and which swelled but did not dissolve in benzene. Addition of alcohol converted it to a white powder.

(*b*) *Antimony pentachloride.*—Two drops of undiluted reagent produced at ordinary temperature a violent reaction, forming a pitchy product soluble in benzene. At the temperature of solid carbon dioxide, the result was similar to that produced by stannic chloride at room temperature. Five drops of a 20% solution in chloroform had a similar effect, the product being orange colored.

(*c*) *Antimony trichloride.*—The powdered reagent produced at room temperature a violent reaction with charring; at the temperature of solid carbon dioxide, a result similar to that with stannic chloride.

(*d*) *Ferric chloride.*—Only slight effect after two days.

(*e*) *Ferric bromide.*—Gave a dark brown weak gel after 12 hr.

(*f*) *Aluminium chloride.*—Complete polymerization after 24 hr. to a yellow flaky mass only slightly soluble in benzene.

(*g-i*) *Thorium bromide*, 5 drops boron trichloride in 20% chloroform solution, 5 drops boron tribromide in 20% chloroform solution.—Little or no evidence of polymerization after 24 hr. in any of the three cases. Addition of alcohol after 48 hr. precipitated a small amount of powdery polymer.

When a 3% solution of rubber in benzene was treated with the following catalysts, *viz.*, $SbCl_5$, $SnCl_4$, $SbCl_3$, $AlCl_3$, $FeBr_3$, highly colored complexes were formed, which on the addition of alcohol yielded white or pale-yellow powders, mostly only very slightly soluble.

Similar results to those obtained with isoprene were obtained by adding antimony pentachloride and stannic chloride to dimethylbutadiene.

Polymerization of dimethylbutadiene

Examination of the Oily By-products

In no case did examination of the oily, dimeric polymerization products yield any evidence of the presence of an open-chain dimer analogous to "β-myrcene".

Pure dimethylbutadiene was heated at 85° C. for periods of 101, 120, 154 and 250 hr., the quantity of diene taken being 100 cc. in all cases except the second, where it was 375 cc. Unchanged diene was removed by distillation under ordinary pressure and the oily polymerization products were then isolated by distillation under reduced pressure. The yield of oil from a single experiment was so small that the oil from the four experiments (5-6 cc.) was combined and subjected to examination. It boiled at 80° C./10 mm. and showed n_D^{26}, 1.4775. It gave no precipitate with an aqueous solution of sulphur dioxide. When heated in a sealed tube at 100° C. for four days, it became slightly viscous and colored and the refractive index rose to 1.4910 (26° C.), but there was no evidence of the formation of a caoutchouc, as it gave no precipitate when alcohol was added.

In another experiment 100 cc. of pure dimethylbutadiene was heated at 145° C. for 12.5 hr. and the oily polymer isolated. After purification the oil gave no precipitate with sulphur dioxide solution. The oil yielded a sharply boiling fraction, 198-200° C./748 mm.; n_D^{21}, 1.4799.

The oil obtained in the above experiments is clearly essentially the same as

the dimeric oil (dimethyl dipentene) isolated by Lebedev (17, 21) and by Aschan (1, 3), from the polymerization products of dimethylbutadiene, and by Kondakov (13) and Richards (29) from the decomposition products of dimethylbutadiene caoutchouc, as Table VIII shows.

TABLE VIII
DIMERIC PRODUCTS FROM DIMETHYLBUTADIENE

Substance	Boiling point, °C.	Refractive index (n_D)
Oil from polymerization at 85° C. (*supra*)	Approx. 80 (10 mm.)	1.4775 (25° C.)
Oil from polymerization at 145°C. (*supra*)	198-200 (748 mm.)	1.4799 (21° C.)
Lebedev's compound	205 (750 mm.) 85 (13 mm.)	1.47716 (19.7° C.)
Aschan's compound	200.5-201.3 (760 mm.) 87 (12 mm.)	1.47915
Richards' compound	205 (760 mm.)	1.47786 (25° C.)

Effect of Temperature and Time on the Polymerization

Samples of dimethylbutadiene were heated, in glass tubes sealed at atmospheric pressure, for different periods of time at temperatures of 45°, 85° and 145° C. The yields of oily dimers and of caoutchouc were obtained in a similar way to that used in the corresponding experiments with isoprene. The results have been summarized in Table I.

In all the experiments with dimethylbutadiene recorded in Table I the tubes used were of the same size (105 cc.) and contained the same volume of diene (50 cc.). The total volume in each experiment at 85° and 145° C. was 100 cc.; in the experiment at 45° C., 250 cc., and in the experiment at room temperature, 1000 cc. A further experiment, in which the ratio of air to diene was higher than in the above experiments, showed *inter alia* that the proportion of air to diene influences the rate of polymerization. In this experiment the tubes were 250 cc. in volume and contained 75 cc. of diene. The total volume of diene used was 375 cc. After 120 hr. at 85° C. the yield of caoutchouc polymer was 18.3%.

Change of Refractive Index with Polymerization of Dimethylbutadiene

Samples of 5 cc. of the diene were heated in sealed tubes of 15-cc. capacity for various periods at 85° C. and the refractive index and density of the total product was measured in each case. As the previous experiments have shown that only a small proportion of dimer is formed at 85° C. and that the main polymeric product is methyl rubber, it was possible to calculate the percentage of rubber formed at any time with fair accuracy. According to Macallum and Whitby (22) the refractive index of methyl rubber is 1.5250 (at 20° C.), and that of the monomer from which it was prepared 1.4377 (at 20° C.). The change in refraction for 100% polymerization is thus 0.0873. The monomer used in the experiments recorded in Table IX had n_D^{25} 1.4637.

In the following experiment the effect of air on the rate of polymerization was again observed. A quantity (10 cc.) of dimethylbutadiene was sealed in

TABLE IX

CHANGE IN REFRACTIVE INDEX AND DENSITY WITH THE POLYMERIZATION OF DIMETHYLBUTADIENE

Time at 85° C., hr.	Refractive index, n_D^{25}	Density, d_4^{25}	Rubber formed %
0	1.4367		
12	1.4420	0.7335	
24	1.4435	0.7372	
72	1.4464	0.7397	8.3 (found)
127	1.4486	0.7457	11.2 (found)
186	1.4500	0.7469	
372	1.4540	Too viscous	19.82 (calcd.)
550	1.4590	Too viscous	25.5 (calcd.)
578	1.4590	Too viscous	

each of two tubes of 25 cc. capacity. One tube was sealed in the ordinary way and the other while the tube was cooled with solid carbon dioxide and ether and it was evacuated to 10 mm. The two were heated for 46 hr. at 85° C. The refractive index of the contents was measured, with the following results.

n_D^{26} of liquid in tube filled with air, 1.4440.

n_D^{26} of liquid in tube sealed under reduced pressure, 1.4420.

n_D^{25} of original diene, 1.4367.

Heterogeneity of Dimethylbutadiene Caoutchouc

Methyl rubber (5.5 gm.) was dissolved in 50 cc. of pure benzene, and there was added a mixture of 80 parts of absolute alcohol and 20 parts of benzene until a faint turbidity appeared. On standing overnight, the mixture separated into two layers, the upper one being quite fluid and the lower one very viscid. The layers were separated and the polymer precipitated from each by the addition of absolute alcohol. This gave Fraction 1 and Fraction 2, the latter being the larger. The products were carefully dried *in vacuo*, and the viscosity of a sol prepared from a portion of each was measured. The balance of Fraction 2 was redissolved in benzene and separated into two sub-fractions (2a, 2b) by precipitation with the alcohol-benzene mixture. The viscosity of sols prepared from the fractions was measured. The results are shown in Table X.

TABLE X

TIME OF FLOW OF SOLS (0.8544 GM./25 CC. BENZENE) OF FRACTIONS OF METHYL RUBBER)

Fraction No.	1	2	2a	2b
Time of flow, sec.	55.4	72.1	67.8	81.3

Unsaturation of Dimethylbutadiene Caoutchouc

The polymer prepared by heating dimethylbutadiene at 85° C. for 101 hr. was purified by repeated solution in benzene and precipitation with alcohol and finally by prolonged drying *in vacuo*. The unsaturation of the product was

TABLE XI

VISCOSITY AND MOLECULAR WEIGHT OF DIMETHYLBUTADIENE CAOUTCHOUC SAMPLES

Sample, time and temp. of polymerization	Viscosity		Molecular Weight	
	Conc., gm./20 cc. benzene	Relative viscosity, benzene = 1	Conc., gm./20 cc. benzene	Mol. wt.
(a) 101 hr. at 85° C.	0.0862	1.24	0.2121	1443
	0.1881	1.53	0.5042	2110
	0.3231	1.96	0.7700	3400
	0.5161	2.80		Mean,
	0.6677	3.69		2318
(b) 154 hr. at 85° C.	0.0819	1.27	0.2230	1973
	0.2632	1.87	0.6788	3982
	0.3181	2.15	0.6713	4017
	0.4974	2.93	0.8597	4067
	0.6713	3.82		Mean,
	0.6788	3.83		3345
	0.7147	4.11		
	0.8088	4.64		
	0.8597	5.08		
	0.9076	5.36		
(c) 250 hr. at 85° C.	0.2121	1.78	0.2615	2402
	0.3270	2.25	0.6971	4645
	0.4600	3.00		Mean,
	0.6454	3.93		3524
	0.6971	4.55		
	0.8143	5.17		
	0.8990	6.38		
(d) 900 hr. at 85° C.	0.1529	2.49		
	0.2501	3.49		
	0.4830	7.19		
	0.6770	9.21	0.6770	3206
	0.6811	10.86		
	0.8721	13.20	0.8721	3760
				Mean,
				3483
(e) 12.5 hr. at 145° C.	0.2363	1.41	0.6804	2100
	0.4537	1.81	0.6885	2175
	0.6804	2.30		Mean,
	0.6885	2.34		2138
	0.8625	2.84		
(f) 2628 hr. at 45° C.	0.2418	1.53	0.2418	529
	0.4489	2.06		
	0.5255	2.24		
	0.6883	2.76	0.6883	1923
	0.7315	2.79	0.7315	2102
	0.8570	3.05	0.8570	2294
(g) 1920 hr. at room temp.	0.2435	1.31	0.6838	1700
	0.4705	1.62	0.6612	1799
	0.5345	1.72	0.6922	2278
	0.6305	1.87		Mean,
	0.6813	1.99		1926
	0.7477	2.11		
	0.7961	2.23		
	0.9031	2.33		

determined by the method of Kemp (11). The iodine numbers obtained were 309.1, 307.8, 298.6 and 297. These numbers correspond sufficiently well with the calculated value of 309.1 to show that the polymer contains, as nearly as such a method of analysis can decide, one double bond for each (C_6H_{10}) unit.

Viscosity and Molecular Weight of Dimethylbutadiene Caoutchouc Samples

The rubber-like residues left after removing unchanged diene and oily polymers in the above experiments were purified by precipitation as in the case of the corresponding isoprene caoutchouc samples, and determinations were similarly made of the molecular weight of the samples and the viscosity of their sols. The results are shown in Table XI. Mean values for the molecular weights have been recorded in Table I.

Change in Viscosity of Sols on Keeping

It was found that sols of dimethylbutadiene caoutchouc showed a marked decrease in viscosity when kept. In Table XII are recorded measurements made after keeping a number of sols in the dark for various periods, the volume of each sol being 20 cc. and that of the containing bottles 50 cc. (cf. Fig. 2).

TABLE XII

CHANGE IN VISCOSITY OF SOLS OF DIMETHYLBUTADIENE CAOUTCHOUC ON KEEPING

No.	Conc., gm./20 cc. benzene	Time of flow (sec.) at 30.1° C.		
		8 days after preparation	17 days after preparation	33 days after preparation
1	0.8990	155.4	115.0	81.6
2	0.8143	126.2	98.9	73.6
3	0.6454	95.8	78.4	61.6
4	0.4600	73.2	63.4	51.7
5	0.3270	55.0	50.0	43.9
6	0.2121	43.3	41.2	37.7

NOTE:—*Time of flow of pure benzene: 24.4 sec.*

As the above data show, the higher the original viscosity the greater is the decrease; further, the rate of decrease falls as time goes on.

It was found that the change in the viscosity of the sols resulted from a permanent change in the polymer. Each of two sols which had been kept for 53 days was poured into absolute alcohol. As the precipitates would not

TABLE XIII

No.	Conc., gm./20 cc. benzene	Relative viscosity at 30.1° C.		
		(a) Fresh sol.	(b) After keeping 53 days	(c) After recovering rubber from (b) and redissolving it
1	0.8088	4.65	2.38	2.40
2	0.7147	4.23	2.23	2.23

coagulate, the mixture was evaporated at room temperature, and the product, which was sticky and entirely different from the originally tough, elastic material, was made up to the original concentration in fresh benzene. The viscosities of the solutions, measured next day, agreed, not with the initial but with the decreased values, as the figures in Table XIII show.

The fall in viscosity is apparently due to oxidation; it could be prevented by the addition of dimethylaniline, as is shown in Table XIV in which are recorded the results with a sol of dimethylbutadiene caoutchouc (obtained by polymerization at 45° C.) in benzene.

TABLE XIV

	Time of flow, sec.	
	Originally	After 34 days
Without addition	49.2	40.1
With 0.0926 gm. dimethylaniline in 20 cc. sol.	49.2	49.3

The behavior on vulcanization of some of the rubber polymers described herein will be dealt with in a forthcoming paper.

Addendum. There has come to the authors' attention since the above was written a paper by T. Wagner-Jauregg (33) in which also the alleged formation of open-chain dimers on heating isoprene is traversed experimentally, with results in substantial agreement with those reported in the present paper. Following polymerization conditions given by Harries and conditions given by Ostromislenski, no open-chain dimeric product was formed. On heating isoprene at 85-90° C. for 4-5 days the main terpenic fraction had the following properties:— b.p. 170-174° C., $n_D^{21.5}$, 1.4730. It was not polymerizable on heating with sodium. The author regards it as identical with Aschan's diprene, but did not examine it for dipentene. On heating isoprene (160 cc.) with acetic acid (0.8 cc.) at 100° C. for six days the main fraction had the following properties:— b.p. 172-174.5° C., n_D^{17}, 1.4754; d_4^{19}, 0.8454. This material too, which corresponds to Harries' product, is also regarded as being identical with Aschan's diprene. The author remarks that "diprene" is probably a mixture of closely similar hydrocarbons. The author points out that the recent recognition of the frequent occurrence of the isoprene skeleton in nature, no only in terpenes, sesquiterpenes, and rubber, but also in carotinoids, squalene* phytol, bile acids, cholesterol, hop resin acids, confers added interest on the question of the modes of dimerization of isoprene.

Acknowledgment

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Cf. References 35 and 36.

References

1. ASCHAN, O. Ber. 57: 1959-1962. 1924.
2. ASCHAN, O. Ann. 439: 221-232. 1924.
3. ASCHAN, O. Ann. 461: 1-26. 1928.
4. F. BAYER & Co. Brit. Pat. 17,734 of 1910.
5. BOUCHARDAT, G. Bull. soc. chim. 24 (2): 111-114. 1875.
6. BRUSON, H. A., SEBRELL, L. B. and CALVERT, W. C. Ind. Eng. Chem. 19: 1033-1037. 1927.
7. GUSTAVSON, G. and DEMJANOFF, N. Chem. Centr. 2: 1345-1346. 1888.
8. HARRIES, C. D. Ber. 35: 3256-3266. 1902.
9. HARRIES, C. D. Ann. 383: 157-227. 1911.
10. HARRIES, C. D. and GOTTLOB, K. Ann. 383: 228-229. 1911.
11. KEMP, A. R. Ind. Eng. Chem. 19: 531-533. 1927.
12. KONDAKOV, I. J. prakt. Chem. 64: 109-110. 1901.
13. KONDAKOV, I. Rev. chim. pure et appl. 1912, 168. (See Dubosc, A. and Luttringer, A. Rubber: its production, chemistry and synthesis in the light of recent research. Griffin & Co., London. 1918. p. 333).
14. KONDAKOV, I. Annales de l'Université de Jergew, 1900-1902. (See Dubosc. A. and Luttringer, A. Rubber: its production, chemistry and synthesis in the light of recent research. Griffin & Co., London. 1918. p. 332).
15. KONDAKOV, I. Le Caoutchouc, 15: 9466. 1918.
16. KYRIAKIDES, L. P. J. Am. Chem. Soc. 36: 987-1005. 1914.
17. LEBEDEV, S. V. J. Russ. Phys. Chem. Soc. 41: 1868. 1909.
18. LEBEDEV, S. V. J. Russ. Phys. Chem. Soc. 42: 949-960. 1910. (Chem. Centr. 2: 1744. 1910).
19. LEBEDEV, S. V. J. Russ. Phys. Chem. Soc. 45: 1249-1295. 1913. (Chem. Abstr. 9: 798. 1915).
20. LEBEDEV, S. V. J. Russ. Phys. Chem. Soc. 45: 1296-1331. 1913.
21. LEBEDEV, S. V. and SKAVRONSKAIA, N. A. Chem. Centr. 1: 1002. 1918.
22. MACALLUM, A. D. and WHITBY, G. S. Trans. Roy. Soc. Can. 18:III: 191-193. 1924.
23. MACALLUM, A. D. and WHITBY, G. S. Trans. Roy. Soc. Can. 22:III:34-44. 1928.
24. MOKIEWSKY, W. Chem. Centr. 1: 589. 1899.
25. MUNTWYLER, O. Thesis, Zurich, 1917. (See Schotz, S. O. Synthetic rubber. E. Benn, London. 1926, p. 94)
26. OSTROMISLENSKI, I. I. Ger. Pat. 276,185, March 29, 1913.
27. OSTROMISLENSKI, I. I. Fr. Pat. 475,565. July 22, 1914. (See J. Soc. Chem. Ind. 35: 130. 1916).
28. OSTROMISLENSKI, I. I. J. Russ. Phys. Chem. Soc. 47: 1911-17; 1928-31. 1915.
29. RICHARDS, A. H. Compt. rend. 153: 116-120. 1911.
30. SCHOTZ, S. P. Synthetic rubber. E. Benn. London. 1926.
31. STAUDINGER, H. Ber. 59: 3019-3043. 1926.
32. TILDEN, W. A. J. Chem. Soc. 45: 410-420. 1884.
33. WAGNER-JAUREGG, T. Ann. 488: 176-185. 1931.
34. WALLACH, O. Ann. 227: 277-302. 1885.
35. WHITBY, G. S. Trans. Inst. Rubber Ind. 5: 184-195. 1929.
36. WHITBY, G. S. Trans. Inst. Rubber Ind. 6: 40-62. 1930.
37. WHITBY, G. S. and KATZ, M. J. Am. Chem. Soc. 50: 1160-1171. 1928.
38. WHITBY, G. S. and KATZ, M. Can. J. Research, 4: 344-360. 1930.
39. WHITBY, G. S. and KATZ, M. Can. J. Research, forthcoming publication.
40. WHITBY, G. S., MCNALLY, J. G. and GALLAY, W. Trans. Roy. Soc. Can. 22:III:27-32. 1928.

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THE MORPHOLOGY OF THE HEAD-CAPSULE OF SOME COLEOPTEROUS LARVAE[1]

BY W. E. WHITEHEAD[2]

Abstract

A comparative study of the external morphology of 46 species of the more common coleopterous larvae. The antennae and ocelli exhibit wide variation. From the fronto-dorsal aspect, the generalized structure of the head is shown, with which may be compared the modifications which occur and which lead to the disappearance of all the sutures separating the different areas found in this region. From the postero-ventral aspect, the heads are divided into two distinct groups, those that possess a gular area and those that do not. Here again, the specialization is compared with the generalized condition. Where a gula occurs, its development is first traced through the series, and then its gradual suppression, due to specialization, is illustrated. The disappearance of sutures causes the head-capsule to become very compact and heavily sclerotized, and this condition is found to be more general and complete in the campodeiform type of larvae.

Introduction

The original purpose of this study was to obtain some data on the phylogeny of the heads of coleopterous larvae. This has not been very successful owing to the difficulty in obtaining long series of any genus or family necessary for such a study. For this reason the subject is discussed from a comparative standpoint. As the order Coleoptera is very extensive and shows a great diversity of form, a long period of time would be necessary to cover more than a small portion of it. There are but 30 families represented in the following pages, but these show what diverse conditions obtain in the head-capsules of the larvae.

Both campodeiform and eruciform larvae occur in the Coleoptera. The former is a term taken from *Campodea*, a genus in the order Thysanura, which represents an early stage in the phylogenetic development of insects and is the more primitive type. Individuals in this group are usually quite active, with horizontal mouth parts and elongate, flattened bodies. The eruciform larvae on the other hand are not as active, the mouth parts are vertical and the body is more or less cylindrical. The form is usually corre-

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lated with the insect's habits. Campodeiform larvae are mostly predacious, which accounts for their activity and great development of the mandibles, whereas the eruciform larvae are usually surrounded by an abundance of food from the time they hatch from the eggs, so that very little activity is necessary. There are also modifications of the eruciform type as in the scarabaeid larvae which develop very heavy bodies and show still less activity. In addition to these there are the apodous larvae found among the boring beetles, in which case there is no need of much movement.

Although the campodeiform larvae are the most primitive from an evolutionary standpoint, they show the greatest specialization as far as the sclerites of the head-capsules are concerned. These structures in the eruciform group are of a very much more generalized nature. Whatever the degree of specialization may be, however, it bears no relation to the condition found in the adult insects, but is an adaptation dependent upon the requirements of the insects during their larval life.

There has been no large amount of work done on the comparative anatomy of coleopterous larvae. With few exceptions, the available literature deals with conditions existing within a family, or even a smaller group. Probably among the most comprehensive are the works of Gage (8), "Larvae of the Coccinellidae," and Hayes (11), "Morphology, Taxonomy and Biology of Larval Scarabaeoidea."

The following discussion, although on a very small scale, will at least give some idea of the wide variation in structure that exists among the larvae in the more common families of the Coleoptera.

List of Species

The following were the available species. They are not arranged in any particular order and the numbers are given only for convenience.

1. *Harpalus honestus*, Carabidae 2. *Carabus nemoralis*, Carabidae 3. *Cicindela* sp., Cicindelidae 4. *Dytiscus* sp., Dytiscidae 5. *Hydrophilus obtusatus*, Hydrophilidae 6. *Pseudophonus pubescens*, Erotylidae 7. *Cantharis* sp., Cantharidae 8. *Gnathocerus punctulatus*, Histeridae 9. *Cercyon* sp., Sphaeridae 10. *Silvanus surinamensis*, Cucujidae 11. *Epilachna borealis*, Coccinellidae 12. *Adalia bipunctata*, Coccinellidae 13. *Passalus cornutus*, Lucanidae 14. *Dorcus parellelepipedes*, Lucanidae 15. *Staphylinus* sp., Staphylinidae 16. *Micromalthus debilis*, Lymexylonidae 17. *Aulonium* sp., Colydiidae 18. *Synchita* sp., Colydiidae 19. *Silpha tristis*, Silphidae 20. *Scaphidium 4-maculatum*, Scaphidiidae 21. *Thanasimus formicarius*, Cleridae 22. *Dermestes lardarius*, Dermestidae 23. *Tenebrio molitor*, Tenebrionidae 24. *Rhyncophorus cruentatus*, Curculionidae 25. *Anthonomus grandis*, Curculionidae 26. *Balaninus* sp., Curculionidae 27. *Osmoderma scabia*, Scarabaeidae 28. *Allorhina nitida*, Scarabaeidae 29. *Phyllophaga anxia*, Scarabaeidae 30. *Labidomera clavicollis*, Chrysomelidae 31. *Phyllotreta armorica*, Chrysomelidae 32. *Chelymorpha argus*, Chrysomelidae 33. *Leptinotarsa decemlineata*, Chrysomelidae 34. *Cassida vittata*, Chrysomelidae 35. *Malachius bipustulatus*, Melyridae 36. *Anisandrus*

pyri, Scolytidae 37. *Dendroctonus valens*, Scolytidae 38. *Orchesia micans*, Melandryidae 39. *Scobicia declivis*, Bostrichidae 40. *Chrysobothris femorata*, Buprestidae 41. *Acanthocinus obsoletus*, Cerambycidae 42. *Xylotrechus colonus*, Cerambycidae 43. *Prionis laticollis*, Cerambycidae 44. *Saperda candida*, Cerambycidae 45. *Lampyris* sp., Lampyridae 46. *Agriotes mancus*, Elateridae.

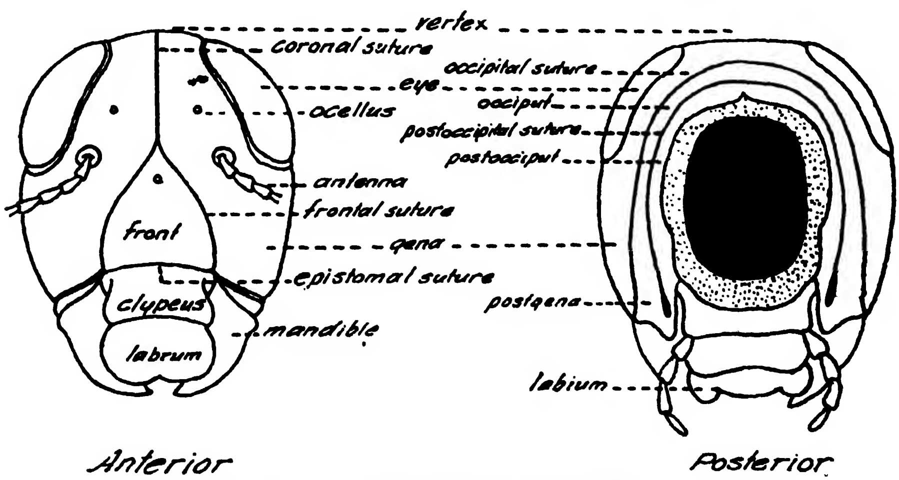
Abbrevations Used in Labelling Figures

acl, anteclypeus
an, antenna
anb, antenna base
ap, accessory process of antenna
as, accessory suture
at, anterior pits of tentorium
bp, basal process of antenna
cll, clypeal-labral area
cm, cervical membrane
cs, coronal suture
edm, edge of cervical membrane
ep, epicranium
epgs, epigular suture
es, epistomal suture
fa, frontal apodeme
fcl, fronto-clypeal-labral area
fpr, fronto-parietal
fr, front
fs, frontal suture
ge, gena
gm, gulamentum
gs, gular suture
gu, gula
lr, labrum
ls, lateral suture
md, mandible
mn, mentum
msm mento-submental area
mx, maxilla
oa, occipital apodeme
oc, ocellus
ocp, occiput
of, occipital foramen
pcl, postclypeus
pg, postgena
po, postocciput
por, postoccipital ridge
pos, postoccipital suture
pr, parietal
pt, posterior pits of tentorium
sm, submentum
sml, submental lobe
smt, submentales
tu, tubercle

Parts of the Head-capsule

Under this heading are included those parts other than the fronto-clypeal and the postero-ventral regions which are dealt with separately.

The accompanying diagrams illustrate the generalized structure of an



General structure of head (modified from Snodgrass).

insect's head. The chitinous walls of the head-capsule constitute the epicranium and this is divided by sutures into various sclerites. The vertex is marked by a median coronal suture that turns downward and divides into the frontal sutures which extend to the anterior articulations of the mandibles. The coronal suture and the frontal sutures constitute the epicranial suture. The median facial region between the frontal sutures is the frons, ventrad to which, and separated from it by the epistomal suture, is the clypeus, with the labrum suspended from the lower margin of the latter.

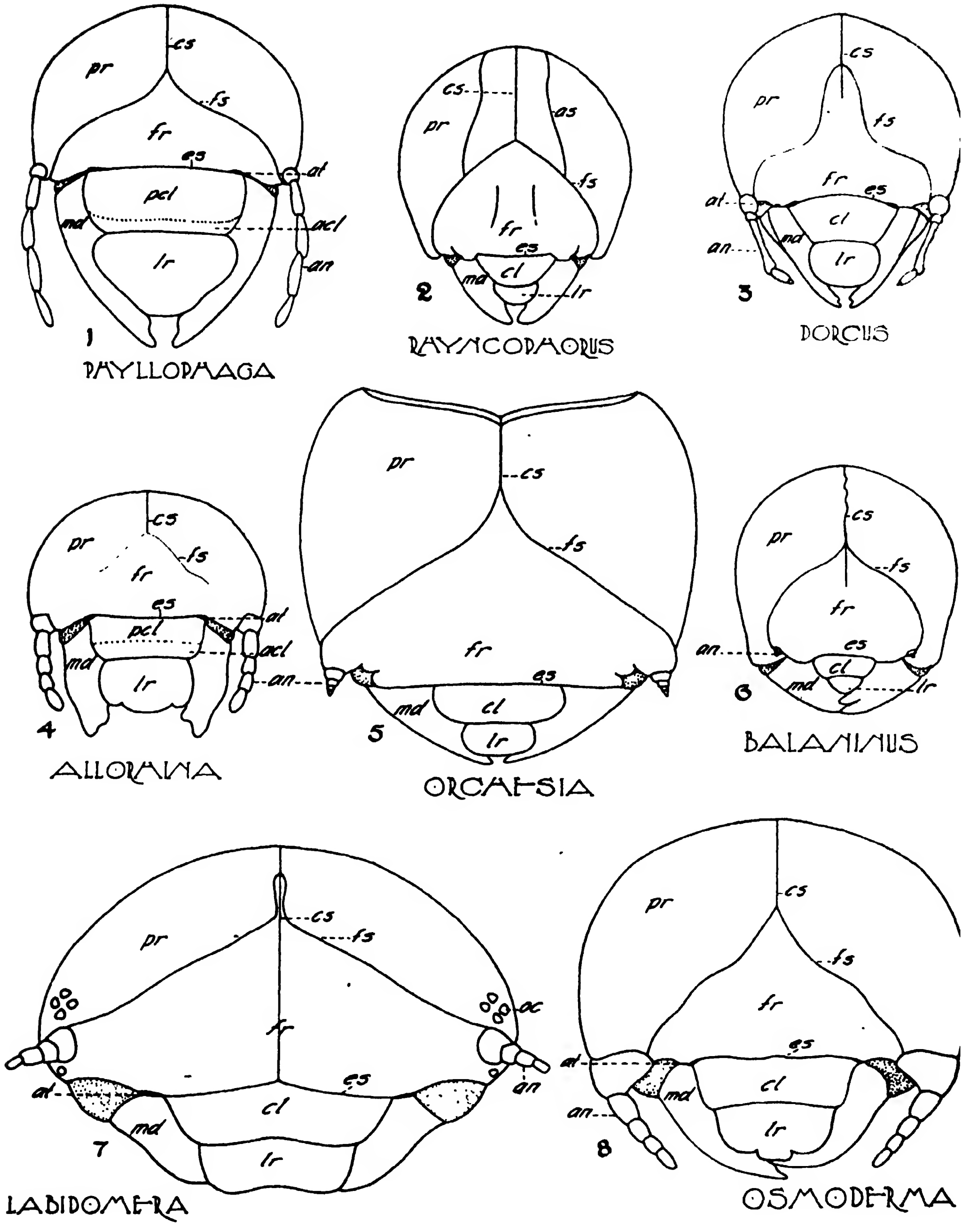
A large portion of the posterior surface of the epicranium is occupied by the occipital foramen. Surrounding this opening dorsally and laterally is the occipital area, the anterior limit of which is defined by the occipital suture. Another suture, the postoccipital, separates the narrow, marginal postocciput to which the cervical membrane is attached. The dorsal part of the occipital area anterior to the postocciput is termed the occiput, and the latero-ventral parts, the postgenae. To the ventral margin of the occipital foramen is attached the labium.

The lateral areas of the epicranium between the occipital suture and the frontal sutures, and separated dorsally by the coronal suture, are termed the parietals. The parietal area behind and below the compound eye is the gena, that between the eyes is the vertex.

In all the more generalized species of larvae studied there is nothing peculiar about the head, it is held more or less vertically with the occipital foramen located posteriorly. As specialization develops, however, we find this condition changed in many groups, the head becomes uptilted so that it is held in a horizontal position with distinct dorso-ventral compression in many species and invariably a change in the position of the occipital foramen. There is no connection between this specialization and that found in adult Coleoptera, in fact a complete reverse condition often exists, as in the case of the adephagous beetles, which are considered to be among the most generalized and have some of the most specialized structures in the larvae. Many of the polyphagous beetles show quite primitive structures in the larval stages.

It will not be necessary to mention many of the species used, as a similar condition exists in all of them, suffice it to say that it is to be noted that in such species as *Dorcus* (Fig. 3) and *Osmoderma* (Fig. 8) to name only two, we find a generalized condition present. Among other head capsules of this type there are a few characteristics which might be mentioned. In *Rhynchophorus* (Fig. 2) for instance, the parietals are crossed by accessory sutures, and in *Pseudophonus* (Figs. 27 and 27A) and in *Hydrophilus* (Fig. 30) there are lateral sutures, neither of which appear to be of any structural importance, although they are the only species among those used in this study which show these characteristics. The posterior margin of the dorsum in *Phyllotreta* (Fig. 20) and *Chelymorpha* (Fig. 23) are deeply notched, while the latter bears on each dorso-lateral margin a group of four tubercles, a characteristic of certain other larvae.

The retraction of the head-capsule within the cervical membrane is a point



FIGS. 1 - 8.

of interest and shows different degrees of development in the series. According to Snodgrass (17), the postoccipital ridge in some larvae develops into an apodemal plate, shown in *Phyllophaga* (Fig. 45) and which is mostly covered by the cervical membrane. This condition is also shown in *Dorcus* (Fig. 47),

Allorhina (Fig. 48), *Osmoderma* (Fig. 52), and, to a lesser degree, in *Rhyncophorus* (Fig. 46).

In another group consisting of *Chrysobothris* (Fig. 12), *Scobicia* (Fig. 13), *Acanthocinus* (Fig. 16), *Xylotrechus* (Fig. 9), *Prionis* (Fig. 15) and *Saperda* (Fig. 11), it is to be noted that the head-capsule is deeply retracted within the cervical membrane. To consider that this enveloped condition of the posterior part of the head is a development of the apodemal plate would seem to be an erroneous conclusion. A more probable explanation would be that it is a further development of that found in *Phyllophaga* where the cervical membrane covers only a comparatively small portion of the postero-ventral surface. As the head assumed a horizontal position, instead of the cervical membrane covering the apodemal plate, gradually receding to the edge of the occipital foramen, it remained stationary, and the dorsal and lateral surfaces of the posterior part of the head-capsule during the course of the uptilting gradually retracted backward into the cervical membrane. This would also account for the occipital foramen being in a ventral position, with the exception of *Chrysobothris*, in which species it is directly posterior but cutting into both dorsal and ventral surfaces.

There seems to be little doubt that the retracted portion of the head-capsule, or at least a large portion of it, was originally exposed. The sclerotization of both areas is similar, and sutures in some species found on the exposed portion can be distinctly traced backward on to the retracted area. Examples of this condition may be seen in *Scobicia* where there is apparently a distinct coronal suture, also in *Saperda* where we find a faint continuation of the coronal suture and a distinct prolongation of the lateral sutures.

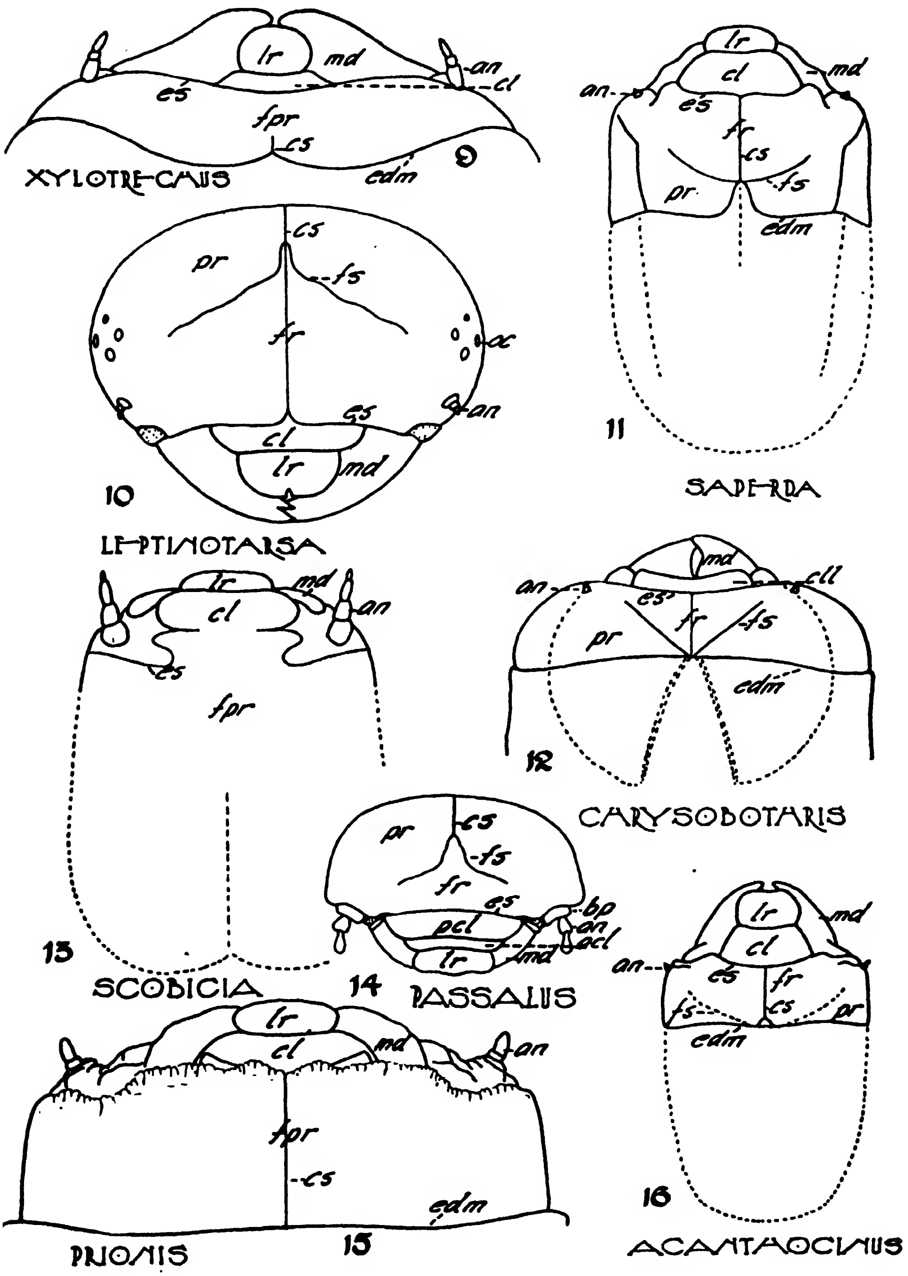
Of species, other than those already referred to, whose head has attained the horizontal position, little more can be said, other than in *Hydrophilus* (Fig. 30) and *Cicindela* (Fig. 36) examples of a dorsal occipital foramen occur.

Ocelli

Ocelli may be present or absent, they may vary in number from one to six pairs (*Hydrophilus*, Fig. 30); there is a wide range in size and their location varies to some extent. They are usually located on the dorso-lateral margin of the parietals near the base of the antennae, although this is not always true, as in *Cicindela* (Fig. 36), where they are situated considerably posterior to the antennae. As a rule they are easily discernible, sometimes attaining a comparatively large size, as in *Dytiscus* (Fig. 33) and *Cicindela*, but may also be minute, as in *Scaphidium* (Fig. 31).

Where more than one pair are present, their grouping is used to some extent in the classification of the larvae. They may be close together and evenly spaced as in *Staphylinus* (Fig. 41) and *Harpalus* (Fig. 32), or separated into groups, a characteristic in *Scaphidium* and *Labidomera* (Fig. 7), or again, they may be close together and arranged on a prominence as shown in *Carabus* (Fig. 34).

It is among the boring larvae and those that have a subterranean habitat, that we find the forms having no ocelli.



FIGS. 9 - 16.

Antennae

As in other characters, the antennae exhibit much variation. They are located in the cephalo-lateral area of the head-capsule, the frontal sutures,

when complete, invariably extending to their bases. There is some departure from their usual location in *Cercyon* (Fig. 44), *Staphylinus* (Fig. 41) and *Hydrophilus* (Fig. 30) where they have migrated medially. There is also some variation as to their proximity to the bases of the mandibles. In such examples as *Silpha* (Fig. 28), *Adalia* (Fig. 39), *Thanasimus* (Fig. 26), *Tenebrio* (Fig. 19), *Harpalus* (Fig. 32) and *Pseudophonus* (Fig. 27), the antennae are situated close to the mandibular articulations, but *Leptinotarsa* (Fig. 10) and *Cassida* (Fig. 43) show them to be situated posterior to the mandibular joints.

Malachius (Fig. 17) shows a unique condition as far as this series is concerned. The antennae are situated in cavities and are probably protrusible, although this point could not be ascertained with the material available.

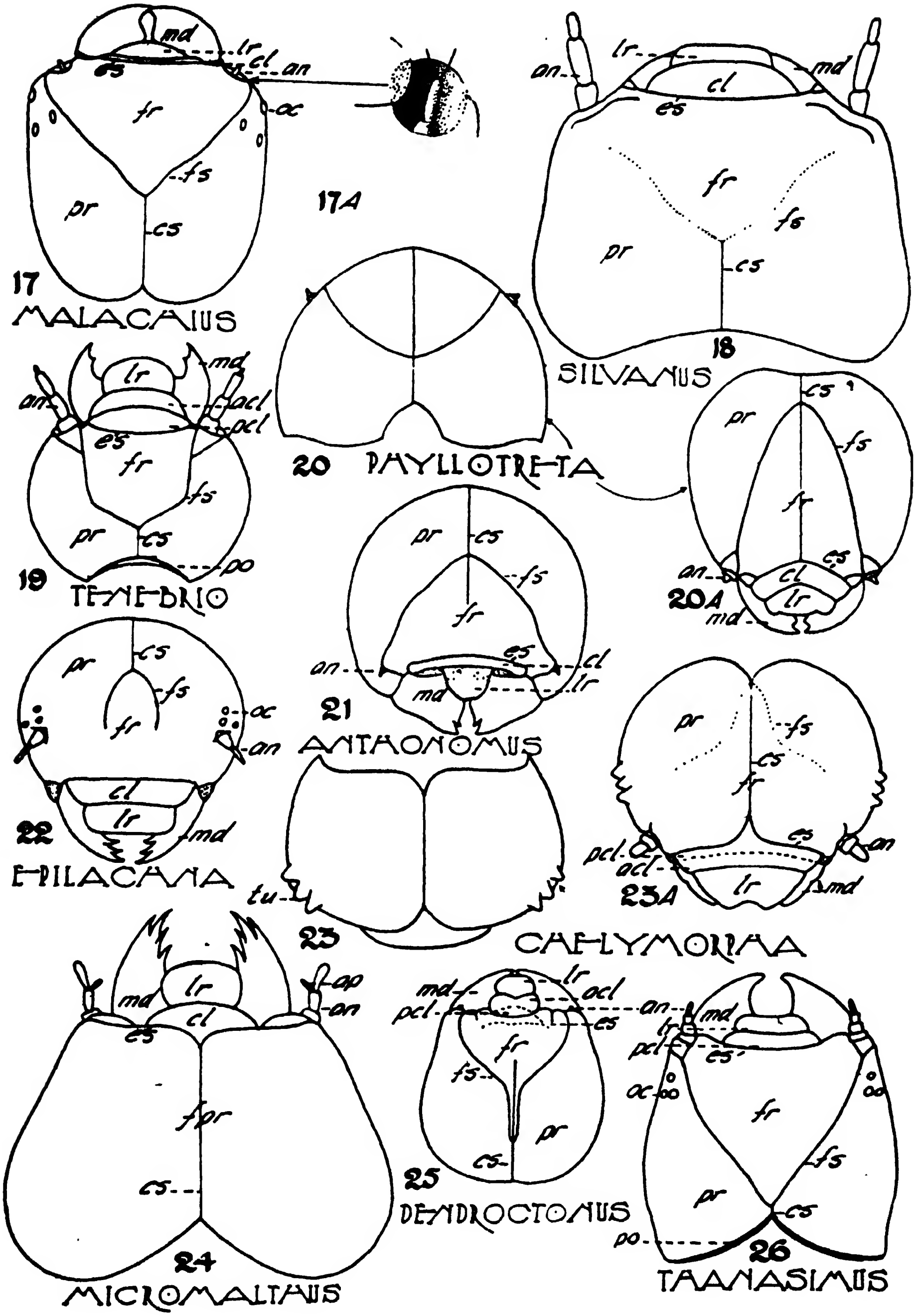
Size shows a very wide range. In boring larvae such as *Anthonomus* (Fig. 21), *Anisandrus* (Fig. 35) and *Chrysobothris* (Fig. 12), to mention only three, the antennae are minute, and from these there is a gradual increase in size to those found in *Silpha* (Fig. 28). Not only is the difference in the length, but there is also a marked difference in diameter, as illustrated when comparing *Silpha* with *Scaphidium* (Fig. 31). There is usually a basal process, well illustrated in *Passalus* (Fig. 14), and the segments attached to that, and which are numbered from the base, may be conical as in *Chelymorpha* (Fig. 23A), and the boring larvae, globular in *Passalus*, or elongate as in *Staphylinus* (Fig. 41), *Silpha*, or *Dorcus* (Fig. 3).

A supplemental process occurs on some antennae, the location of which also varies. In *Micromalthus* (Fig. 24) it is found on the first segment, in *Cercyon* (Fig. 44), *Agriotes* (Fig. 29) and *Aulonium* (Fig. 40) on the second, while the third antennal segments bear them in *Staphylinus*, *Harpalus* (Fig. 32) and *Pseudophonus* (Fig. 27).

The only species which has apparently no antennae is *Rhyncophorus* (Fig. 2) and although a careful examination has been made it has failed to reveal any. The antennae are very inconspicuous in the other boring larvae, but in this species have disappeared altogether.

Fronto-clypeal Region

The fronto-clypeal region includes, typically, the front or frons, that area between the frontal sutures which extends from the coronal suture to the neighborhood of the anterior articulations of the mandibles, and the clypeus, that area ventrad, or cephalad, depending on the angle of the head, to the front. This area is separated from the front by the epistomal suture, a groove coinciding with an internal transverse ridge between the anterior articulations of the mandibles and which strengthens the lower edge of the face. It has been suggested by some writers that the clypeus was an articular region between the front and the labrum and which secondarily developed into a sclerotized plate. Snodgrass (17) however, disproves this by the fact that the most anterior muscles of the stomodeum have their origin upon the inner surface. The clypeus often shows some differentiation in being divided by a



FIGS. 17 - 26.

partial or complete suture into an anteclypeus, a more or less membranous area next to the labrum, and a postclypeus adjacent to the front.

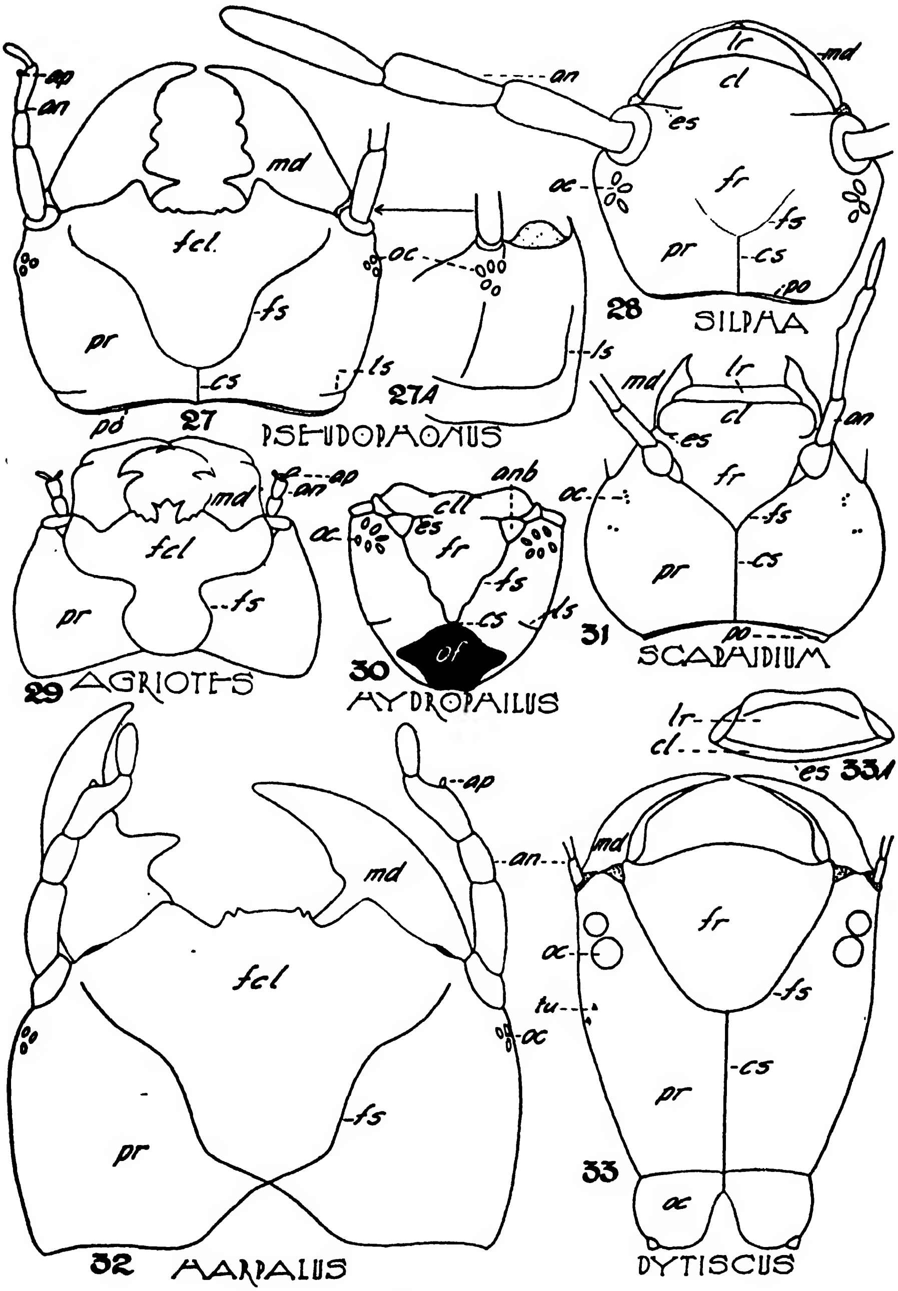
For convenience it might be well at this time to mention the labrum, since in cases where modifications occur in the fronto-clypeal region this area is frequently involved. The labrum, unlike the two preceding parts, is movable, hangs as a free flap before the mouth, and is attached to the anterior margin of the clypeus.

Of the head-capsules under consideration, a typical fronto-clypeal area is singularly well illustrated in *Phyllophaga* (Fig. 1) a species which, as far as this region is concerned, shows a very generalized condition, and on which all the previously mentioned points may be observed. While several other species are as typical, it might be well to use this one as a standard by which the various modifications occurring in other species may be compared.

In order to facilitate analysis, species showing similarities will be grouped together as much as possible. The first group includes *Dendroctonus* (Fig. 25), *Anthonomus* (Fig. 21), *Thanasimus* (Fig. 26), *Osmoderma* (Fig. 8), *Orchesia* (Fig. 5), *Tenebrio* (Fig. 19), *Phyllotreta* (Fig. 20A), *Labidomera* (Fig. 7), *Dorcus* (Fig. 3), *Balaninus* (Fig. 6), *Rhyncophorus* (Fig. 2) and *Malachius* (Fig. 17). All of these species have a full complement of parts in the region under discussion and it will be found that the frontal sutures are fully developed. *Osmoderma*, *Tenebrio* and *Dermestes* are very similar to *Phyllophaga*, but certain slight modifications occur in some of the other species. These are concerned with the coronal suture which extends beyond the union of the frontal sutures on to the front for varying distances. In *Dorcus* it is quite short, somewhat longer in *Balaninus*, *Anthonomus* and *Dendroctonus*, while in *Phyllotreta* and *Labidomera* it extends to the epistomal suture. The clypeus is very suppressed in *Malachius*, but is still intact.

The second grouping consists of *Silvanus* (Fig. 18), *Epilachna* (Fig. 22), *Leptinotarsa* (Fig. 10), *Passalus* (Fig. 14), *Allorhina* (Fig. 4), and *Chelymorpha* (Fig. 23A). In these species too are to be found all the parts of a typical fronto-clypeal region, but the frontal sutures are incomplete. *Passalus*, *Epilachna* and *Leptinotarsa* show distinct but shortened sutures; in *Silvanus* and *Allorhina*, they are becoming indistinct, while in *Chelymorpha* they are only vestigeal. In *Chelymorpha* also, it is to be noted that the frontal sutures originate at the posterior margin of the head and the coronal suture extends to the epistomal suture, the latter modification also being applicable to *Leptinotarsa*. In *Epilachna*, Underhill (19) figures what is apparently a double coronal suture and the frontal sutures closely converging near their distal extremities, all of which are indicated as being quite definite. He also shows a double epistomal suture. Gage (8) does not find this condition existing in the coronal suture or in the epistomal region, neither were they observed in the specimens examined by the writer.

Another group consists of *Scaphidium* (Fig. 31), *Silpha* (Fig. 28), *Cassida* (Fig. 43), *Anisandrus* (Fig.35), *Micromalthus* (Fig. 24), *Aulonium* (Fig. 40) and *Adalia* (Fig. 39), because each shows a tendency towards reduction in sutures, or because there is an apparent complete disappearance of some part as compared with the primitive type. Of these, *Scaphidium* is the only one



FIGS. 27 - 33A.

having complete frontal sutures, those in *Adalia* are almost so and those in *Aulonium*, which is minus a coronal suture, show somewhat less development, while they are only vestigeal in *Silpha*. In *Anisandrus* the sutures have dis-

appeared, leaving only the coronal suture, in which case frons and parietals combine to form the fronto-parietals. A similar condition exists in *Micromalthus* and *Cassida*, but in these, the coronal suture extends to the epistomal suture. Such a condition may be the result of the forward migration of the frontal sutures, but this would not hold with the views of Barber (1) as far as the larval *Micromalthus* is concerned, as he believes this species to have a distinct clypeus, in which case the dividing suture would be the epistomal.

Apart from these modifications of the epicranial suture, all the foregoing species, with the exception of *Cassida*, show that there is a tendency for the epistomal suture to disappear. The suture in *Anisandrus* can be followed throughout its course, but in the remaining four species it has entirely disappeared with the exception of the lateral portions.

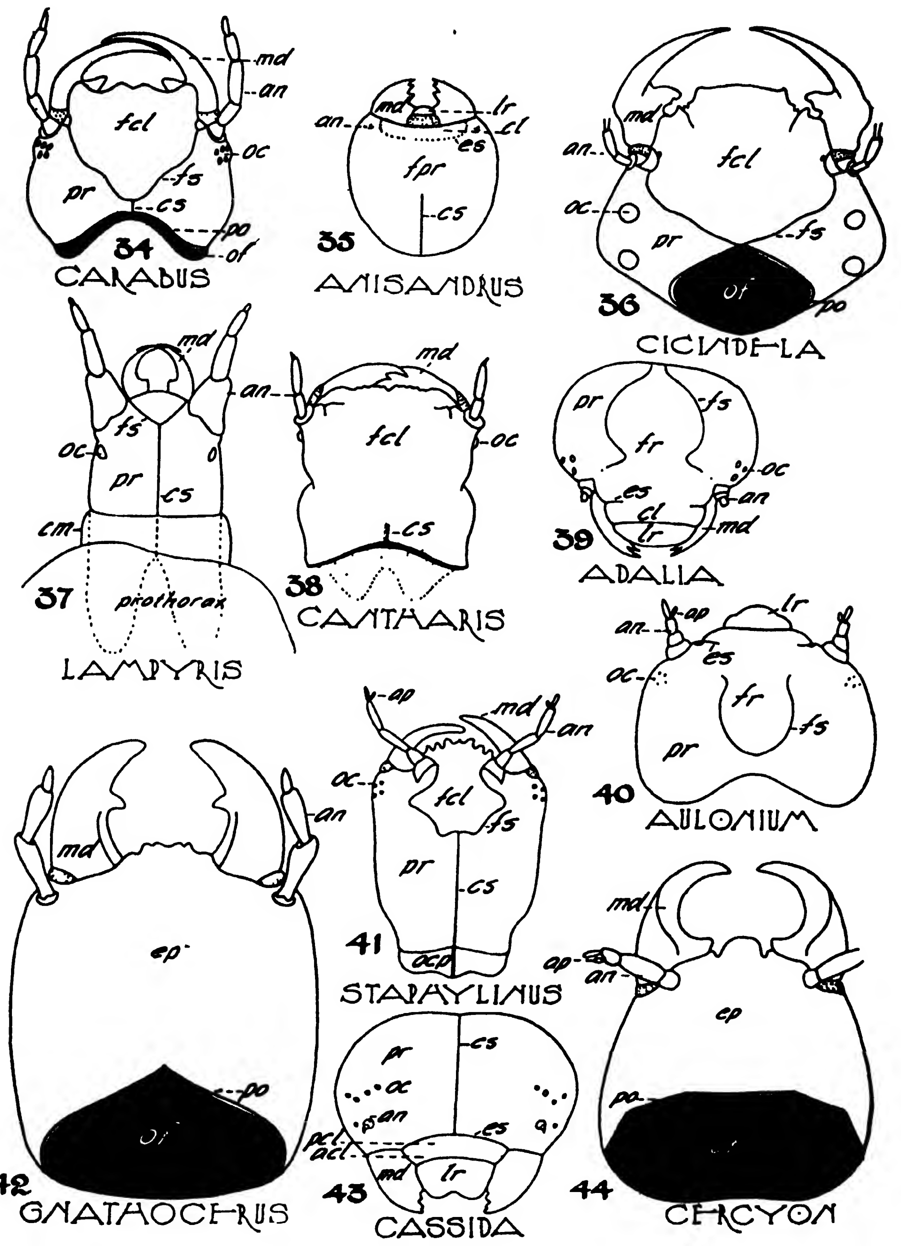
Some of the wood-boring larvae show certain modifications and the following representatives are grouped together on account of the nature of the head-capsule. (*Saperda* (Fig. 11), *Prionis* (Fig. 15), *Xylotrechus* (Fig. 9), *Acanthocinus* (Fig. 16), *Chrysobothris* (Fig. 12) and *Scobicia* (Fig. 13).)

The retraction of the head-capsule into the cervical membrane may have some bearing on the structure of the fronto-clypeal region, as the distance between the margin of the membrane and the anterior extremity of the head becomes considerably shortened. *Saperda* and *Chrysobothris* are perhaps nearest to the typical condition as far as the frontal sutures are concerned, they are not complete in either of the species, but they are least definitely shown. The coronal suture in each instance extends throughout the length of the front. In *Anthocinus* the frontal sutures have almost disappeared and in the remaining species they have done so entirely. The coronal suture persists in *Prionis*, is very reduced in *Xylotrechus* and has completely disappeared in *Scobicia*.

The epistomal sutures are intact in all species except in *Scobicia* and here the median portion is missing. It is to be noted that there is a reduction of sutures in *Chrysobothris*, but it is apparently the suture between the labrum and the clypeus that is absent as, from the position of the tentorial pits, the epistomal suture appears to be intact.

The remaining species studied may be divided into two groups, the larger consisting of *Hydrophilus* (Fig. 30), *Cicindela* (Fig. 36), *Carabus* (Fig. 34), *Harpalus* (Fig. 32), *Pseudophonus* (Fig. 27), *Dytiscus* (Fig. 33), *Lampyris* (Fig. 37), *Staphylinus* (Fig. 41), *Agriotes* (Fig. 29) and *Cantharis* (Fig. 38).

Hydrophilus perhaps shows the least specialization in the fact that there is at least a slight differentiation between the labrum and the clypeus. *Dytiscus* may also be mentioned in this connection as, while no labrum can be seen from the dorsum, this structure may be observed from a cephalo-ventral aspect. *Staphylinus* and *Lampyris* show a fusion of the labrum and clypeus, but in the others, with the exception of *Cantharis* and *Cicadela*, each of which has what are evidently vestiges of sutures, there is no differentiation between the front, the clypeus and the labrum. It is to be noted in all species in this group that the frontal sutures are very well defined and in most instances



Figs. 34 - 44.

complete. A major character in *Agriotes* is that the cephalic margin is tridentate.

The last two species, *Gnathocerus* (Fig. 42) and *Cercyon* (Fig. 44) are the most specialized of all, the epicranium being entirely devoid of sutures.

Cercyon bears two notches on the anterior margin which are probably the remains of some previous suture, and *Gnathocerus* has an extended area at the cephalic margin, which is also notched to a lesser degree, but there are no sutures apparent to differentiate the parts.

From the foregoing discussion it will be obvious that there is a very wide variation of structure in the fronto-clypeal region, ranging from the simple, generalized condition in *Phyllophaga*, to a highly specialized structure as found in such species as *Cercyon* and *Gnathocerus*.

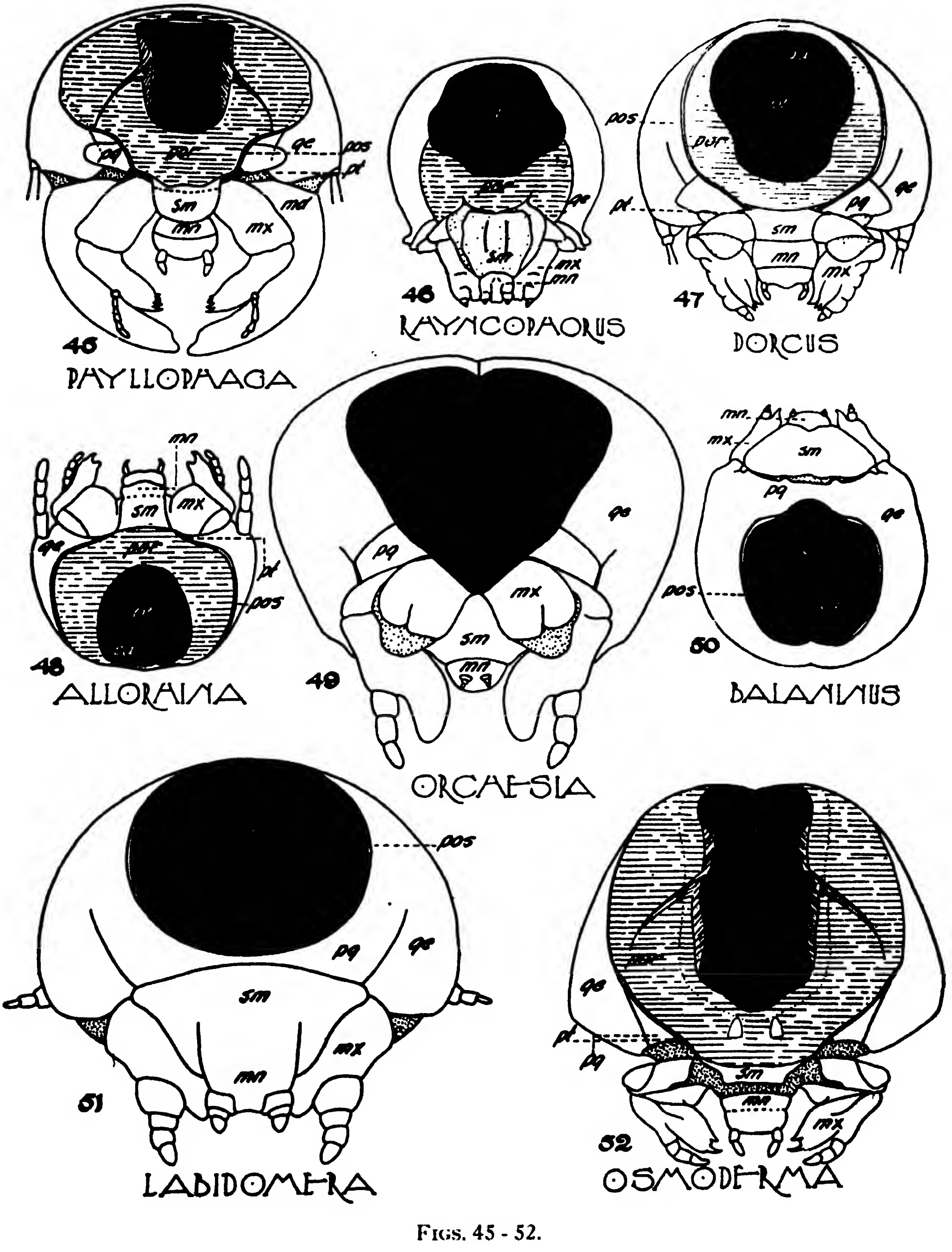
In his work with the head-capsules of adult Coleoptera, Stickney (18) found that in the majority of species, the development trends towards the obliteration of sutures, consequently giving greater compactness and consolidation of sclerites, with a tendency towards stronger sclerotization of the head-capsule. This is all quite applicable to the head-capsules of the larvae. In the eruciform and melolonthoid forms, we have species showing the least amount of sclerotization, but the most complete conditions from a primitive standpoint. At the same time the obliteration of sutures has begun in the disappearance of part or all of the frontal sutures, resulting in a fronto-parietal region, as well as at least the partial disappearance of the epistomal suture, leading towards the formation of a fronto-clypeus.

Among the campodeiform larvae is to be found a gradual increase in specialization, culminating in a complete consolidation of parts. Such species as *Hydrophilus* are probably the most generalized in this group, but they are more highly specialized than the eruciform species, while the other species in the group show a gradual tendency towards the obliteration of sutures. With this obliteration, comes a much greater degree of sclerotization, the cephalic margin of the most highly specialized forms being very marked in this respect. No doubt the habits of the larvae have something to do with the amount of sclerotization they possess, although it seems natural that as the sutures disappear there should be a tendency towards the strengthening of these areas. The degree of disappearance of sutures seems to coincide with the degree of sclerotization, as illustrated in the clypeo-labral area.

Postero-ventral Region

If all the parts of the postero-ventral region of the head-capsule are to be mentioned, they would include the labrum, the maxillae, the postgenae, the occiput, the postocciput, and in some species, the gula. The mandibles also have a ventral or posterior articulation and are frequently observed from this aspect.

The labium consists of a basal part, the submentum, anterior to which is the mentum, to which is attached the eulabium, or what Crampton terms the prementum, which bears the palpi. In the more generalized insects, the labium hinges to the posterior part of the head, completing the ventral margin of the occipital foramen, and on either side of it are to be found the proximal parts of the maxillae, and, depending on the nature of the occipital foramen, the anterior median portions of the postgenae.



FIGS. 45 - 52.

The maxillae are complicated gnathal appendages situated in the membrane on either side of the labium.

The postocciput which is a narrow sclerite surrounding the occipital foramen and the postoccipital suture, which separates it from the occiput, is an important landmark of the head. It is usually present and the posterior tentorial

pits are located in its lower ends; if the pits migrate, the lower ends of the suture become correspondingly longer.

The foregoing parts are shown in *Dorcus* (Fig. 47).

In generalized insects the heads are held vertically, in which case the posterior part of the head is naturally rather short, and while modifications do occur, they are not as profound as in insects possessing a gular region.

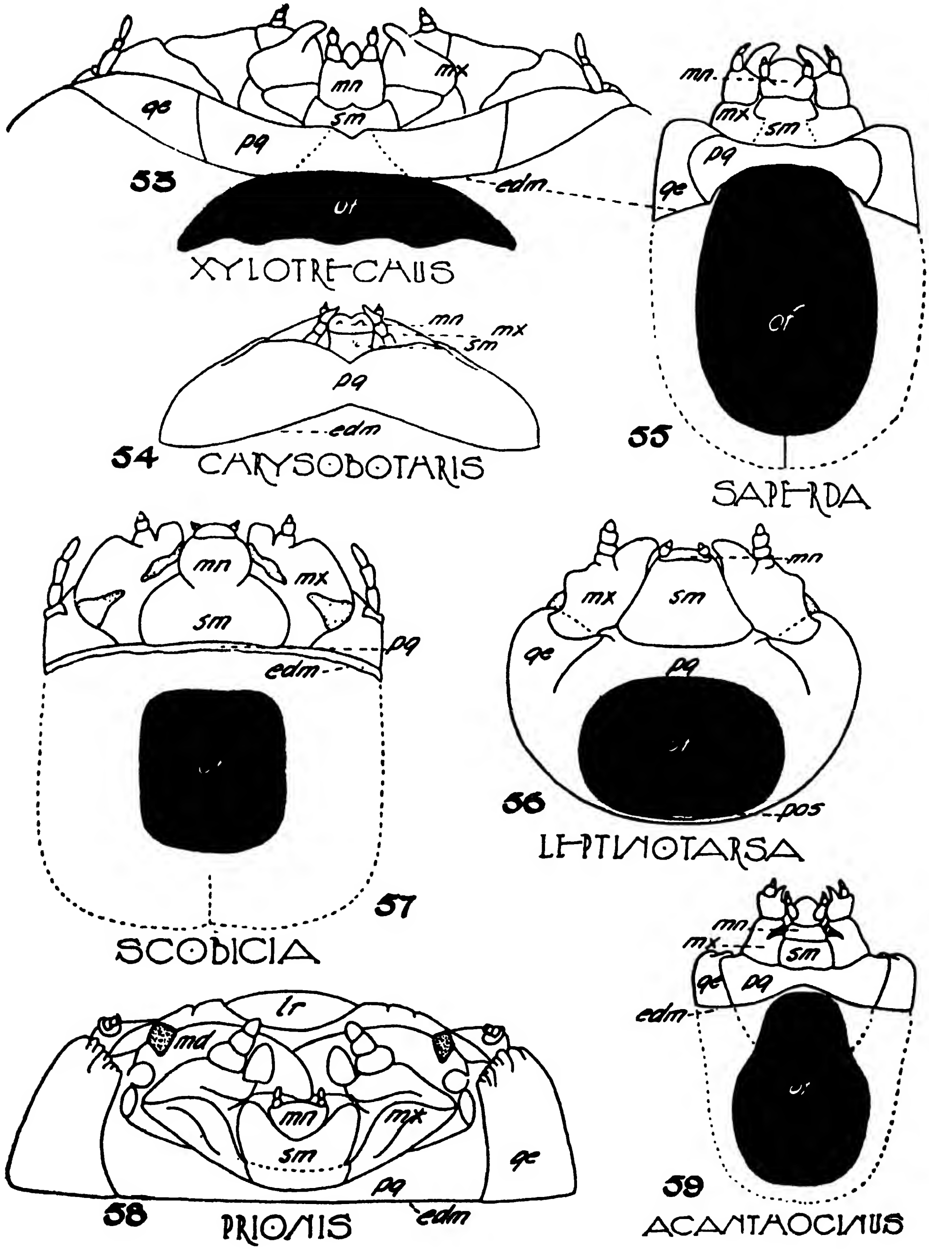
A fully developed gula is found chiefly in those insects whose head is held horizontally, and not always then, and as this condition develops there is a lengthening of the posterior part of the head, the labium moving forward, thus causing the area between the occipital foramen and the submentum to become very much greater.

The origin of the gula has long been a controversial subject. Crampton (7) and later Snodgrass (17) have, however, advanced a theory which seems to be generally accepted, a modified account of which is briefly given here by way of partly explaining the modifications that occur.

In some larvae, *Phyllophaga* (Fig. 45) would be an example of those under discussion, the face is directed forward, the mouth parts hang downward and the under surface of the head is short. The occipital and postgenal regions terminate in a postoccipital suture, in the ventral ends of which are situated the invaginations of the posterior arms of the tentorium. Beyond the suture is a narrow postoccipital rim of the cranium, best developed ventrally, and the postoccipital ridge is developed on each side of the occipital foramen into an apodemal plate, the two uniting ventrally into the tentorial bridge. The basal part of the submentum is sclerotized to form a triangular plate which is attached to the mesal points of the postgenae and has its extreme basal angles prolonged to points behind the tentorial pits.

In *Silpha* (Fig. 70), the general structure of the head is similar, but it will be observed that the ventral postgenal margins are much longer and the posterior tentorial pits are drawn towards the medial line in the prolonged basal angles of the postgenae. The base of the submentum is narrowly constricted behind the tentorial pits, which almost cut off a distinct proximal area, the lateral angles of which become continuous with the postoccipital rim.

The extreme basal area of the submentum is the beginning of the gula which reaches various degrees of development in different species. It is that area between the posterior margin of the submentum and the occipital foramen, the distal extremity of which may be designated as the pregula. Externally, the gula may be marked off anteriorly by the tentorial pits (gular pits), although Crampton has advocated that a line drawn from the basal articulation of one cardo to the other might be preferable to using such shifting landmarks as the gular pits. With the uptilting of the head and the necessary prolongation of the ventral area, the gular pits tend to move forward, under which conditions the gula would become proportionately longer. The gula is bounded laterally by the gular sutures, on or near which are situated the gular pits. On either side of the gula are the postgenae, or what are termed the paragulae by



FIGS. 53 - 59.

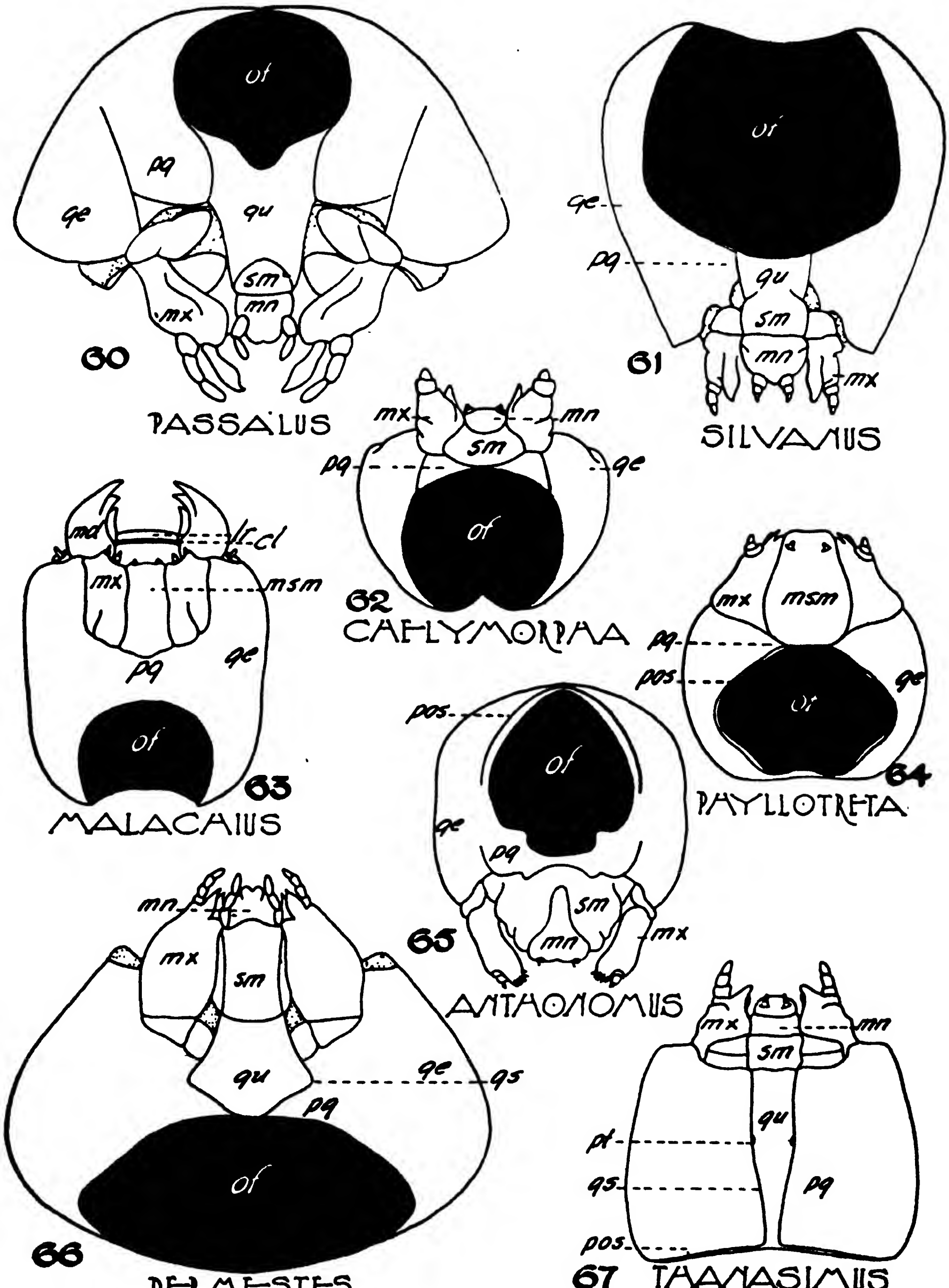
Crampton, who also differentiates the anterior portion as being the hypostoma.
Concerning the head-capsules that are to be considered, it is not difficult to divide them into two groups, namely, those that possess a gula and those that

do not; it is difficult, however, to make further subdivisions owing to the general similarity that exists among many of the species. An effort will be made to group those of different types together, beginning with those that appear to be the more generalized.

The species *Phyllophaga* has already been mentioned in connection with the development of the gular region and there are three other species which resemble this one in respect to the ingrowth of the cervical membrane. *Phyllophaga* and *Osmoderma* (Fig. 52) are very similar, except that the occipital foramen is much larger in the latter. The apodeme is quite extensive in both and it is strengthened by heavily sclerotized crossbars. The only differences appear to be a more complex maxilla in *Osmoderma* and a submentum that is separated from the mentum by a membranous area. *Dorcus* (Fig. 47) and *Allorhina* (Fig. 48) show some similarity in this region and might be placed together, the most notable differences being the apparent absence of a postgenal suture in *Allorhina* and a more extended submentum. Occipital apodemes are present in both species. *Rhyncophorus* (Fig. 46) might also be grouped here as it has some points in common with the others; the ingrown area, however, does not surround the occipital foramen, but extends only over the ventral and part of the lateral margins. An occipital apodeme is present, and the submentum is large, but compared with the surrounding parts of the head-capsule, it is much less heavily sclerotized, the condition being indicated in the figure by slight stippling.

Six species consisting of *Leptinotarsa* (Fig. 56), *Anthonomus* (Fig. 71), *Balaninus* (Fig. 50), *Labidomera* (Fig. 51), *Chelymorpha* (Fig. 62) and *Malachius* (Fig. 63) may be grouped together as having one characteristic in common *i.e.*, the postgenae have grown together forming a bridge of varying width between the occipital foramen and the submentum, and this, together with the tentorial bar, which may be found fused to part of the ental surface, forms a heavily sclerotized plate, adding very considerably to the strengthening and bracing of this area of the head-capsule and resembling very closely the condition found in Lepidoptera. This bridge is quite wide in *Malachius*, *Leptinotarsa* and *Balaninus* and is narrowest in *Chelymorpha*. In all species with the exception of *Malachius*, the submentum shows great development, at the expense, in most cases, of the basal parts of the maxillae and the mentum, which is greatly reduced. In *Malachius* the labium and maxillae show a comparative decrease in size and their situation is more caudad, probably due to the fact that the occipital foramen is more posterior in location. This condition causes the labrum, clypeus and even part of the front, to be considerably anterior to the cephalic margin of the labium and maxillae. The mentum and submentum are apparently fused. The postoccipital area is not well developed in most of the foregoing species, the suture being indiscernible in some. An occipital apodeme occurs in *Balaninus* and *Anisandrus*, the latter also having a frontal apodeme.

Dendroctonus (Fig. 76) might be included here as it somewhat resembles the preceding species. The mentum is well developed, but the submentum, called



FIGS. 60 - 67.

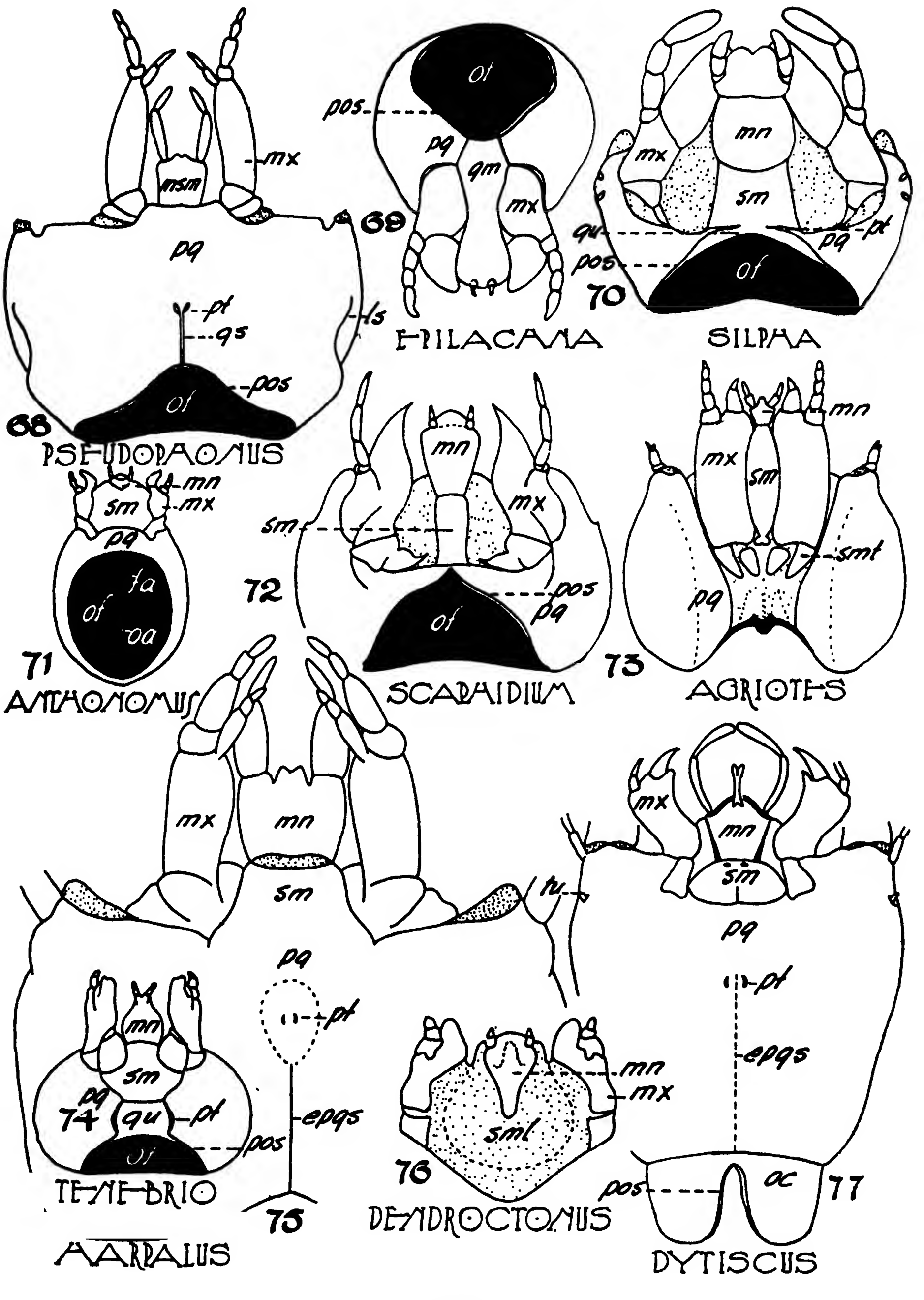
the submental lobe by Hopkins (12), is partly membranous and is separated from the maxillae and the occipital foramen by a membranous area

The members of the group, whose heads are retracted within the cervical membrane, also have a fusion of the postgenae between the occipital foramen and the submentum. In *Scobicia* (Fig. 57) this area is practically covered by the cervical membrane and in *Acanthocinus* (Fig. 59), it is partially covered. In *Chrysobothris* (Fig. 54), *Xylotrechus* (Fig. 53), *Prionis* (Fig. 58) and *Saperda* (Fig. 55), however, it is fully exposed. The mentum and submentum are distinct in all species, and other than the retraction of the head and the ventral occipital foramen, points which have already been discussed, there is nothing characteristic about this group.

Two other species, *Phyllotreta* (Fig. 64) and *Orchesia* (Fig. 49) are very similar to the foregoing species, but they lack the postgenal bridge already referred to, which makes the posterior margin of the submentum adjacent to the occipital foramen without any gular development. This is a similar structure to what one would expect in such species as *Phyllophaga* and *Osmoderma* were it not for the development of the postoccipital ridge, the cervical membrane, in the case of the two foregoing species, being attached to the edge of the occipital foramen.

Still two other species, *Agriotes* (Fig. 73) and *Lampyris* (Fig. 83) are different from all the others. *Agriotes*, whose head is held horizontally and whose occipital foramen is directly posterior, has a membranous area between the submentum and the occipital foramen and between the most developed portions of the postgenae. The cardines of the maxillae are small sclerotized areas in the membrane, and besides these, are two other isolated areas which Crampton (6) terms the submentales. In addition there are two slightly sclerotized areas joined to the posterior margin, which might be the remains of a more sclerotized area. The submentum is very elongate, likewise the maxillae, and the mentum is quite definite. The second species, *Lampyris*, is not identical with *Agriotes*, but has some resemblance in that it also has a posterior membranous area. A possible explanation of this, however, is that the head of this species is retracted within the prothorax, attached to which is a collar, or fold, only slightly sclerotized, which assists in the mechaism of this function. This does not mean that the head could not be retractile if it were heavily sclerotized, but, as the posterior part of the head is never exposed, but attached to the collar which also acts as a protection, there is no need of heavy sclerotization. The submentum is elongate, somewhat like that in *Agriotes*, and while no definite submentales are present, there is a pair of thickenings in the same position as these areas in *Agriotes* which may correspond to them and which are surrounded by a dotted line in the figure.

It is also possible to separate, to some extent, the species of larvae that possess a gula. *Silpha* (Fig. 70) has already been considered in connection with the development of this region and it will be seen that the gula occupies only a small area, but that the submentum, mentum and prementum are all well developed. In *Tenebrio* (Fig. 75), *Dermestes* (Fig. 66), *Scaphidium* (Fig. 72), *Passalus* (Fig. 60) and *Thanasimus* (Fig. 67), the gula is well developed, being very elongate in the last-mentioned species, with decided evidence



FIGS. 68 - 77.

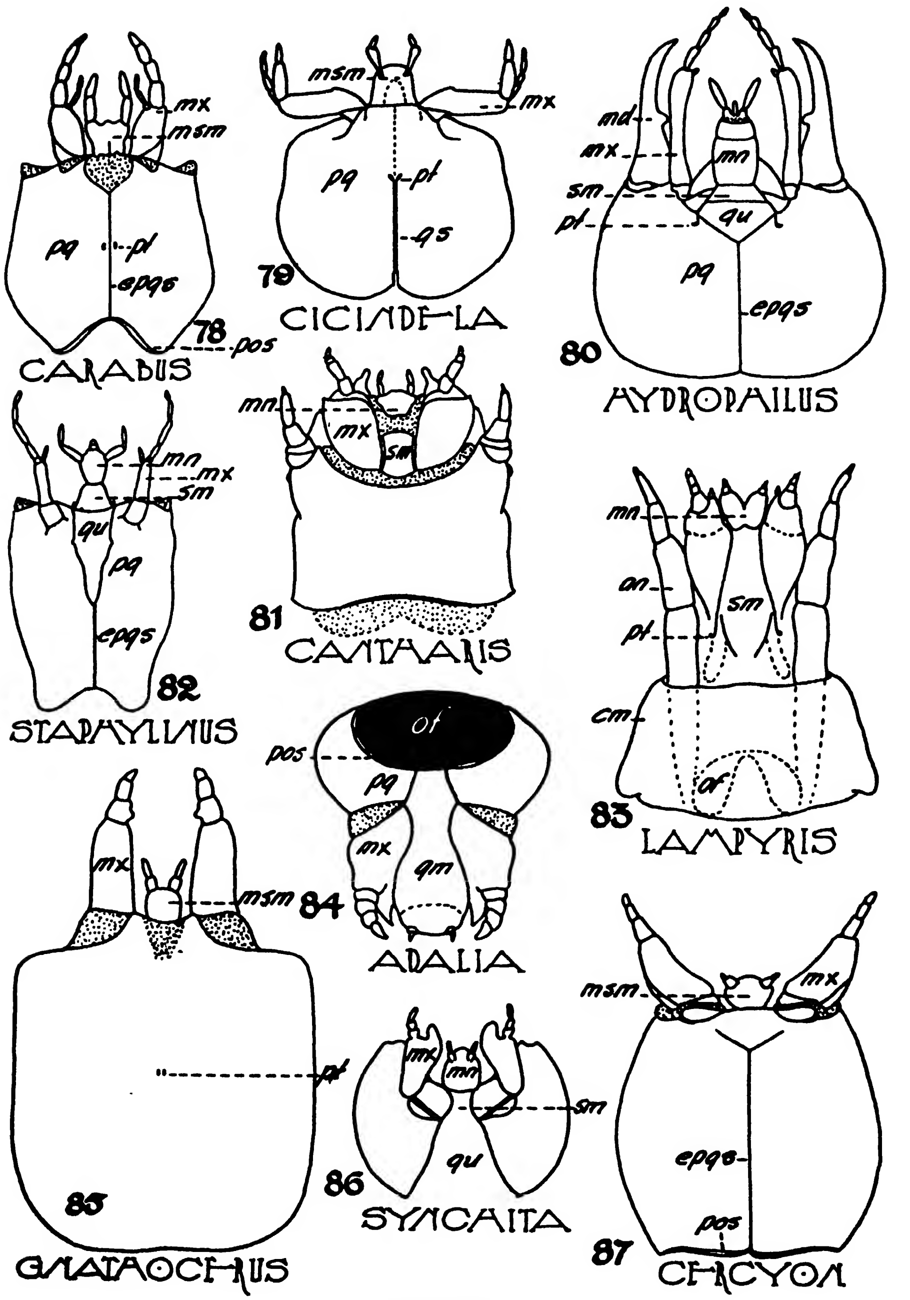
of the tendency of the posterior ends of the gula sutures to become approximated. *Silvanus* (Fig. 61) has a distinct gula, but the suture between it and

the submentum has started to disappear. This disappearance is complete in *Synchita* (Fig. 86), *Adalia* (Fig. 84) and *Epilachna* (Fig. 69) resulting in the formation of a gulamentum. Gage (8) figures a distinct suture between the gula and submentum in *Epilachna*, but in the specimens examined not a vestige of one was visible, neither was the suture separating the submentum from the mentum apparent.

A different condition occurs in *Staphylinus* (Fig. 82) and *Hydrophilus* (Fig. 80), the approximation of the posterior parts of the gular sutures, referred to as commencing in *Thanasimus*, has, in these two species, become complete, forming a single median suture, called by Crampton (7) the epigular suture. He explains this condition as being due to the fact that the gula in this area has become infolded, and suggests the name of epigulae for those parts of the postgenae which become approximated over it. This area is the more extensive in *Hydrophilus*, but in each instance the visible portion of the gula is situated anterior to the epigular suture. The labium in each species is normal, but the maxillae are worthy of mention. It will be seen that the stipites are very long, as are the galeae, while the laciniae are very much reduced. Altogether these appendages more nearly resemble antennae than gnathal appendages.

The remaining species are highly specialized and it is difficult to interpret the parts accurately. *Cercyon* (Fig. 87) and *Carabus* (Fig. 78) appear to be the most generalized of this group and are very similar. The epigular suture extends throughout the length of the ventral surface of the head, with a small pregular area at its posterior extremity; this is membranous in *Carabus*, which is probably a secondary development and, although the gular sutures are complete in this species, they are beginning to disappear in *Cercyon*. The mentum and submentum are fused in both species. *Cicindela* (Fig. 79) probably comes next from the standpoint of specialization. In this species, the pregula has disappeared, but the gular sutures remain definite as far as the gular pits, and are only faintly indicated from that point to the margin of the submentum. In this case the gular sutures have retained their identity but have practically become fused. *Dytiscus* (Fig. 77) shows still further development in that the anterior portion of the epigular suture has quite disappeared and that portion posterior to the gular pits is very indistinct. This species possesses a well-defined submentum, also a very definite occipital suture. *Harpalus* (Fig. 75) and *Pseudophonus* (Fig. 68) show some similarity. The gular sutures are separate in the latter and fused in the former but, while they are definite, they are very much shorter than in the preceding species, which indicates that the gula has been forced out still further, leaving a greater unbroken area in that region of the ventral surface. There is some development between the bases of the maxillae, especially in *Harpalus* which is possibly the remains of the submentum, although there is no suture separating it from the postgenae.

The greatest specialization occurs in *Cantharis* (Fig. 81) and *Gnathocerus* (Fig. 85), as in these species there is no indication of any gular sutures, the ventral surface being entirely unsegmented. The gular pits were observed in



FIGS. 78 - 87.

Gnathocerus and a small membranous area has developed in the postgenae adjacent to the labium, the maxillae are reduced to a minimum, so that the great degree of specialization gives the ventral surface a very simplified

appearance. The gnathal appendages in *Cantharis* show greater development and give a more generalized appearance, but so far as the fixed parts are concerned, there is little difference between the two species.

Any conclusions that may be drawn from the preceding discussion on the postero-ventral region of the head-capsule must be along the same lines as those given in connection with the fronto-clypeal region, namely, that there is a strong tendency towards the obliteration of sutures and a greater consolidation of parts. It must be borne in mind that there are two distinct types of heads among the species studied, those that have a gula and those that do not. The gradual changes towards specialization, so striking in the former, are not as noticeable in the latter, so far as the postero-ventral region is concerned, as the occipital foramen, in many species, occupies a large area, and the fact that the head is generally shorter does not allow or require much modification. There are, of course, exceptions to this but, as a general rule, the greatest modifications occur where a gula is developing, or where one is disappearing.

It is interesting to note how the gula is believed to develop and then to follow its gradual disappearance, which is fairly completely illustrated in this paper, beginning with the fully developed gula and the gradual drawing together of the gular sutures until they finally meet in an epigular suture, first at the posterior margin and later throughout the entire length of the ventral surface, until the gula has been forced out. Following this, the suture gradually disappears, resulting in the fusion of the postgenae into one solid piece covering the entire ventral surface of the head-capsule. The sclerotization is not as heavy on the ventral surface as it is on the dorsal, but as the sutures disappear, there is naturally a greater compactness, and this, in most cases, corresponds in the different species to the modifications that are at the same time occurring in the fronto-clypeal area.

In some insects, it is believed that certain sclerites force their way in between the base of the labium and the cervical membrane which later develops into the gula. The theory with regard to its formation in the Lepidoptera and Hymenoptera, is that the inner angles of the postgenae become separated, fuse and form the gula. This theory is the one advanced by Stickney (18) with regard to the development of this area in the Coleoptera. He believes that it is formed by the migration of the invaginations of the posterior arms of the tentorium from the occipital foramen towards the submentum, and must therefore be derived from the postgenae. In this migration, are produced the gular sutures, between which is the gula.

The view which Snodgrass (17) takes, however, and which has already been referred to, namely, that the gula originates from the submentum, is more recent and the one which finds most favor at the present time.

Summary

1. Only a limited amount of work has been done on the comparative anatomy of the head-capsules of coleopterous larvae.

2. A very wide variation of structure occurs among the species studied.

3. This variation ranges from a very generalized condition in such species as *Phyllophaga*, to a condition showing great specialization, as in the genus *Gnathocerus* and others. Whatever the degree of specialization, however, there is no relation between it and the condition found in the adults.

4. Antennae were present in all species with the exception of *Rhyncophorus*. They were comparatively long and conspicuous in some species and very minute in others.

5. Ocelli may be present or absent and differ both in number and position.

6. As a general rule, the more generalized condition was found among the eruciform type of larvae.

7. Among the campodeiform species the greatest specialization occurs. This is, in some measure, due to the fact that the head is held in a more or less horizontal position.

8. The general trend of specialization is towards the obliteration of sutures and a greater compactness and consolidation of the head-capsule.

9. Coincident with loss of sutures there is a tendency towards greater sclerotization.

10. While other conditions undoubtedly occur among species that have not been studied, they would come within the two extremes of structure found among species dealt with in this paper.

Acknowledgments

The writer wishes to express his appreciation to Dr. E. Melville DuPorte of Macdonald College for his instruction and for his criticisms and suggestions during the course of this work. He also wishes to thank Dr. G.W. Underhill of the Virginia Agricultural Experiment Station, for providing him with specimens of *Epilachna borealis*, Dr. A. W. Rymer Roberts of the Molteno Institute, for representatives of the families Staphylinidae and Cantharidae, and Dr. C. W. Collins of the U. S. Bureau of Entomology, for specimens in the genera *Calosoma* and *Carabus*.

References

1. Barber, H. S. Observations of the life history of *Micromalthus debilis* Lec. (Coleoptera). Proc. Ent. Soc. Wash. 15:31-38. 1913.
2. Boving, A. G. Description of larvae of Bostrichidae and of *Scobicia declivis* Leconte. U.S. Dept. Agr. Bull. 1107. Appendix, 49-54. 1922.
3. Boving, A. G. and Champlain, A. B. Larvae of North American beetles of the family Cleridae. Proc. U.S. Nat. Mus. 57:575-649. 1921.
4. Cotton, R. T. and St. George, R. A. The meal worms. U.S. Dept. Agr. Tech. Bull. 95. 1929.
5. Craighead, F. C. Biology of some Coleoptera of the families Colydiidae and Bothrideridae. Proc. Ent. Soc. Wash. 22: 1-13. 1920.
6. Crampton, G. C. The sclerites of the head, and the mouthparts of certain immature and adult insects. Ann. Ent. Soc. Am. 14: 65-110. 1921.
7. Crampton, G. C. The eulabium, mentum, submentum and gular region in insects. J. Ent. Zool. 20: 1-18. 1928.
8. Gage, J. H. The larvae of the Coccinellidae. Ill. Biol. Monog. 6, No. 4, 1920.
9. Hamilton, C. C. Studies on the morphology, taxonomy and ecology of the larvae of holarctic tiger-beetles (family Cicindelidae). Proc. U.S. Nat. Mus. 65, Art. 17. 1925.

10. Hayes, W. P. The epipharynx of lamellicorn larvae (Coleop.), with a key to common genera. Ann. Ent. Soc. Am. 21: 282-306. 1928.
11. Hayes, W. P. Morphology, taxonomy and biology of larval Scarabaeoidea. Ill. Biol. Monog. 12, No. 2. 1929.
12. Hopkins, A. D. The genus Dendroctonus. U.S. Dept. Agr. Bur. Ent. Tech. Ser. Bull. 17, Pt. 1. 1909.
13. Hyslop, J. A. The phylogeny of the Elateridae based on larval characters. Ann. Ent. Soc. Am. 10: 241-263. 1917.
14. Richmond, E. A. Studies on the biology of the aquatic Hydrophilidae. Am. Mus. Nat. Hist. Bull. 42: Art. 1: 1-93. 1920.
15. Roberts, A. W. R. A key to the principal families of Coleoptera in the larval stage. Bull. Ent. Research, 21: 57-72. 1930.
16. St. George, R. A. Studies on the larvae of North American beetles of the subfamily Tenebrioninae with a description of the larva and pupa of *Merinus laevis* (Olivier). Proc. U.S. Nat. Mus. 65, Art. 1. 1925.
17. Snodgrass, R. E. Morphology and evolution of the insect head and its appendages. Smithsonian Misc. Coll. 81. No. 3. 1928.
18. Stickney, F. S. The head-capsule of Coleoptera. Ill. Biol. Monog. 8. No. 1. 1923.
19. Underhill, G. W. The squash lady-bird beetle. Va. Agr. Exp. Sta. Bull. 232. 1923.

A NEW SPECIES OF PHOMOPSIS[1]

By H. T. Güssow[2] and W. R. Foster[3]

Abstract

In the fall of 1930, both authors independently isolated the identical fungus from lesions of a characteristic stem-end hard rot of potatoes. The fungus belongs to the genus Phomopsis and as it is materially distinct from any known species in details of artificial and natural growth, it is proposed to establish it as a new species. The authors further collaborated in studying the taxonomy of the fungus. A description of the disease caused by this organism and the results obtained from a study of its pathogenicity and physiology will be published at a later date.

Morphology of Fungus

Pycnidia (Figs. 1, 2, 5) within a stroma; mature stroma prominent, innate at first, later erumpent, 0.25-0.50 × 1-2 mm. in size; dothideoid, sessile; broad base, cushion to variable in shape; single, occasionally confluent; sclerotial. At first like a typical sclerotium; coal black, rough externally, soft to cartilaginous, not brittle, easily sectioned; interior: macroscopically black; microscopically towards apex with series of dark-colored thick walled cells, towards base these are fewer. Substance hyaline, cellular. When spore formation takes place upper portion of stroma dividing into several irregular ridge-like, separate cavities; as absorption takes place forming into one cavity, very irregular and typically dothideoid. Hymenium lining entire walls of cavity. Towards hymenium the hyaline color changes into a pale fuscous tint. Finally more or less indistinctly papillate. Papillae modestly beaked and developed anywhere over the surface of the stroma. Cavities filled with conidia, later discharging through ostiole in form of milky white bead, (Fig. 5) "A" spores (of Diedicke's) shown in Fig. 4.

Conidia (Figs. 3, 4 and 5) length $10.72 \pm 0.96\ \mu$, modal length $11.85\ \mu$, minimum length $7.11\ \mu$, and maximum length $13.03\ \mu$, width $4\text{-}6\ \mu$, hyaline, one-celled, spindle shaped, guttulate. Conidiophores (Fig. 5A) prominent persistent, subulate, septate at base, $15\text{-}18 \times 1.5\ \mu$, simple. Stylospores were found only on two occasions, once on a diseased lesion of the tuber and once in a culture on cooked barley seed. These spores, which resemble the "B" spores of Diedicke, were filiform, curved or straight, and measured $8\text{-}30 \times 0.5\text{-}1.5\ \mu$ (Fig. 5 C).

Type on leaves and tubers of *Solanum tuberosum* L. Vancouver, British Columbia, October, 1930, Coll. H. S. MacLeod, District Plant Disease Inspector, Division of Botany, Dominion Department of Agriculture.

Phomopsis tuberivora Güssow et Foster. sp.n.

Pycnidiis in stromate immersis; stromate maturo conspicuo, 0.25-0.50 × 1-2 mill., dothidioideo, sessili, base lata, forma variabili, nigro, singulari, nonnunquam confluente, sclerotioideo.

[1] *Manuscript received February 24, 1932.*
Contribution from the Division of Botany, Experimental Farms, Ottawa, and the Provincial Plant Laboratories, British Columbia.

[2] *Dominion Botanist, Dominion Department of Agriculture, Ottawa, Canada.*

[3] *Assistant Plant Pathologist, British Columbia Department of Agriculture.*

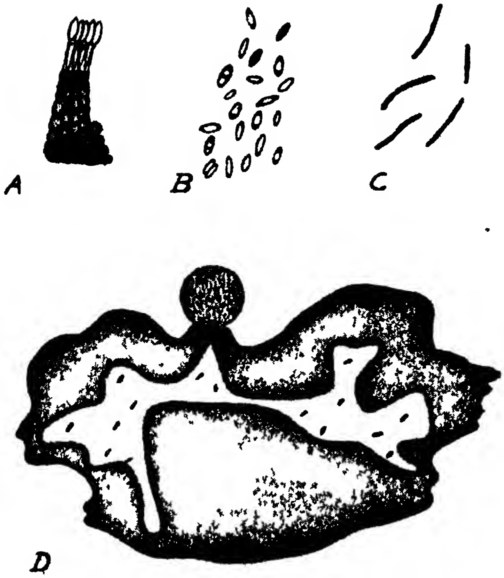


FIG. 5. *Camera lucida drawings: A—Conidiophores bearing conidia, "A" spores. B—Conidia, "A" spores. C—Stylospores, "B" spores. D—Cross section of pycnidium, conidia discharge through ostiole in milky white bead "A" spores.*

Conidiis, 1-cellularibus, hyalinis, fusiformibus, guttulatis; 10.72 ± 0.96 μ × 4-6 μ.

Conidiophoris prominentibus, non deciduis, subulatis, basibus septatis, 15-18 μ × 1.5 μ, simplicibus.

Stylosporis, 1-cellularibus, filiformibus, curvatis vel rectis, 8-30 μ × 0.5-1.5 μ.

Hab. in foliis et tuberibus *Solani tuberosi* L., Vancouver, Columbia Britannica, America borealis.

Discussion

W. B. Grove (2, p. 68) described a new species of Phomopsis found by A. D. Cotton on dead stems of *Solanum tuberosum* in Hampshire, England, to which he gives the specific designation, *P. solani*. In general generic characters our fungus agrees with that of Grove's. The fruiting bodies, however, of our species, are up to 2 mm. long, 1 mm. broad and ½-1 mm. in depth. Further, the spores measure almost double that given by Grove and are frequently multi guttulate. The conidiophores of *Phomopsis solani* are 10-15μ, whereas in our specimen they were 15-18 μ in length. Furthermore it was isolated from living specimens of tubers of *Solanum tuberosum*, collected from several districts of British Columbia. None of the other species agree even remotely with this fungus, we therefore propose to name it *Phomopsis tuberivora*, Güssow et Foster, sp. n.

References

1. Diedicke, H. Die Gattung Phomopsis. Ann. Mycologici, 9 : 8-35. 1911.
2. Grove, W. B. Kew Bul. Misc. Inform. No. 2: 49-73. 1917.
3. Harter, J. J. Fruit-rot, leaf spot, and stem blight, of the eggplant caused by *Phomopsis vexans*. J. Agr. Research, 2: 331-338. 1914.

FIG. 1. *Pycnidia of Phomopsis tuberivora developing on lesion of potato tuber.* (*Photo J. B. McCurry.*)

FIG. 2. *Photomicrograph of cross section of pycnidium of Phomopsis tuberivora.* × 60.

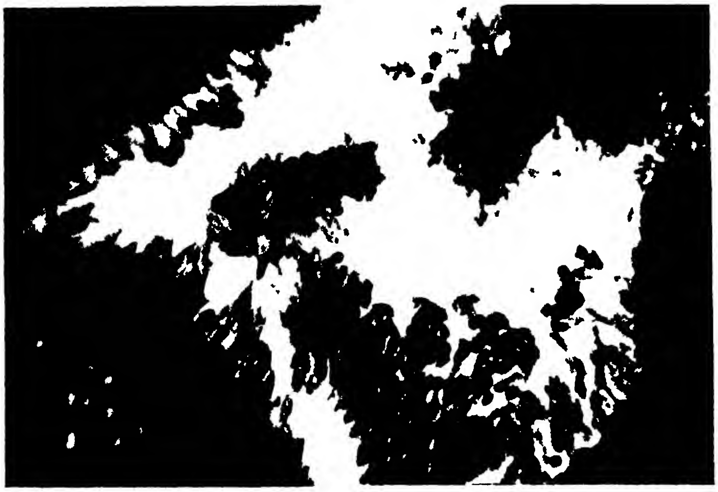


FIG. 3 *Photomicrograph of conidia within a cavity of the pycnidium of Phomopsis tuberivora* × 400

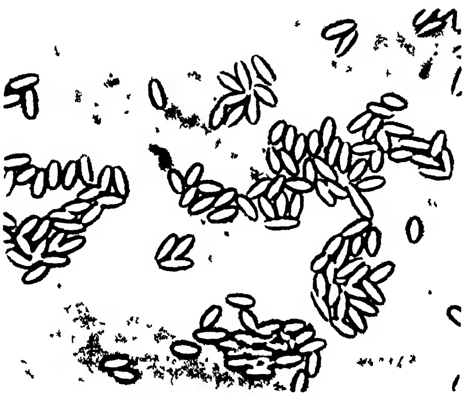


FIG. 4 *Photomicrograph of conidia, "A" spores of Phomopsis tuberivora* × 400

REACTION OF FLOUR OF SOME VARIETIES OF HARD RED SPRING WHEAT TO BLEACHING AGENTS[1]

By R. K. Larmour[2], W. F. Geddes[3] and J. G. Malloch[4]

Abstract

Composite samples of "aged" flour of 17 varieties of spring wheat grown in western Canada, were bleached by three methods, namely, ¾ oz. Betachlor per bbl., 1 lb. Novadel per 40 bbl., and ½ oz. Betachlor per bbl. followed by 1 lb. Novadel per 40 bbl. Gasoline color values were determined for the bleached and unbleached samples and it was found that the more highly pigmented flours in the series could be reduced to approximately the same color as the less pigmented samples, indicating that the former respond to a greater extent to bleaching than the latter. The color of the bread was improved in all cases, the greatest improvement occurring with Betachlor plus Novadel and the least with Betachlor alone. Loaf volumes obtained with six different baking formulas showed no significant difference between bleached and unbleached samples. None of the dosages showed any evidence of overbleaching. It was thought that information might be obtained on the relative susceptibility of the varieties to damage by bleaching agents, but it was found that normal dosages are not sufficient for differentiation on this basis. It is suggested that this might be accomplished by using heavier dosages of Betachlor.

In the domestic trade, flour color and the ease or difficulty of reducing it by bleaching are important factors in the evaluation of varieties. One of the first objections raised by Canadian millers against Garnet wheat was that it produces a yellow flour that cannot be bleached satisfactorily. Other varieties, notably Parker's Selection and Marquillo, have been discriminated against for the same reason. There are available many good varieties of hard red spring wheat of satisfactory agronomic characteristics and hence it is not surprising that any new introduction may be condemned if it fails to compare favorably with the standard variety, Marquis, on even a single count. It is necessary, therefore, when making comparative studies of varieties of one class to consider flour color and reaction to bleaching as well as the baking strength. Malloch, Geddes, and Larmour (8) have conducted an extensive comparative study of the milling and baking quality of the principal varieties grown in western Canada. As residues of the flours used in that study were available it was thought advisable to conduct a special investigation of flour color, bleaching, and the effect of bleaching agents on loaf volume.

Materials

There were available from the 1929 Variety series (8) certain flour residues from which were chosen 17 varieties, each grown in 10 different places. The 10 samples of each variety were thoroughly mixed, giving 17 composite flours each containing aliquots representing the same 10 places of origin. Larmour

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[3] *Professor of Agricultural Chemistry, University of Manitoba.*
[4] *Assistant Research Biologist, Associate Committee on Grain Research, University of Alberta.*

and Brockington (6) have shown that composites made of samples of one variety from different places give results in good agreement with the average values obtained from the tests of the individuals. It was considered, therefore, that the composites would give a very fair estimate of the general reaction of the varieties for study. The varieties were Kitchener, Preston, Red Fife, Dicklow, Garnet, Red Bobs 222, Reward, Marquis, Marquillo, Hard Federation, Early Triumph, Axminster, Renfrew, Supreme, Huron, Kota, and Parker's Selection. They were grown in Edmonton, Lethbridge, Lacombe, Swift Current, Indian Head, Rosthern, Saskatoon, Scott, Winnipeg and Brandon. After being thoroughly mixed, the flours were subdivided into three parts, one of which was sent to each of the collaborating laboratories, where they were halved, one-half being retained as a check sample and the other being bleached. The bleaching methods used in the different laboratories were:— Laboratory A; Novadel at the rate of 1 lb. per 40 bbl.; Laboratory B, Betachlor at the rate of ¾ oz. per bbl.; Laboratory C, Betachlor ½ oz. per bbl. followed by Novadel at the rate of 1 lb. per 40 bbl. Color of the bleached and unbleached samples was estimated by the tentative method of the Association of Official Agricultural Chemists (1, p. 176), using high grade gasoline for the extraction. Comparisons were made against a 0.005% solution of potassium chromate in a suitable colorimeter.

The advantages of this method of estimating color are that it is rapid and requires no intricate or expensive apparatus. It has the disadvantage that the reference color standard consisting of 0.005% potassium chromate is not entirely satisfactory inasmuch as the hue does not exactly match the hue of a gasoline solution of carotin. Jorgensen (4), Kent-Jones and Herd (5), and Visser't Hooft and de Leeuw (9) have suggested various modifications of the color standard, but none of these have proved entirely suitable. Use of a color analyzer has been shown by Ferrari and Bailey (3) to give the best results but the spectrophotometers on the market at present are exceedingly expensive and no such apparatus is available in any of the collaborating laboratories. As this is merely a preliminary study of color it was decided, therefore, to use the chromate standard in order to ascertain if the results obtained would warrant a more thorough investigation of this phase of flour testing.

In dealing with the effects of bleaching agents, a very useful figure is obtained by calculating the ratio of the gasoline color values of the bleached and unbleached flours. This ratio gives a convenient measure of the reduction in color and is valuable in comparing the effectiveness of various bleaching materials and also in comparing the response of different varieties or samples to the same bleaching agent. The ratio of the bleached and check samples can be determined also directly in the colorimeter by using one of the extracts in place of the chromate color standard. This procedure has the advantage that the colors vary only in intensity and should therefore obviate errors attributable to difficulties in matching the chromate standard. The usefulness of such values is strictly limited as they give no indication of the actual color value of the flour, but represent only the relative changes in color intensity.

However, if the ratios obtained by these two different methods agree, one may assume that the error due to matching of hues is not large. A comparison of these ratios was made in two of the laboratories and the results in Table I

TABLE I

RATIOS BLEACHED TO UNBLEACHED COLOR VALUES DETERMINED BY CALCULATION FROM THE SEPARATE GASOLINE COLOR VALUES AND BY COMPARING THE TWO EXTRACTS DIRECTLY IN THE COLORIMETER

Variety	Gas. color value (against K_2CrO_4)		Ratio B/C calcu-lated	Ratio B/C det'm'd. directly	Gas. color value		Ratio B/C calcu-lated	Ratio B/C det'm'd. directly
	Check	Bleached (Nov.+Cl_2)			Check	Bleached (Betachlor)		
1. Kitchener	1.54	0.50	0.33	0.35	1.52	0.96	0.63	0.68
2. Preston	1.52	0.45	0.30	0.28	1.01	0.86	0 86	0.82
3. Red Fife	1.25	0.50	0.40	0.44	1.36	1.10	0.81	0.83
4. Dicklow	1.22	0.34	0.28	0.38	1.62	1.52	0.94	0.89
5. Garnet	1.25	0.47	0.38	0.38	1.21	1.12	0.92	0.88
6. Red Bobs	1.10	0.31	0.28	0.31	1.43	1.16	0.81	0.77
7. Reward	1.03	0.40	0.39	0.35	1.32	1.06	0 80	0.75
8. Marquis	1.10	0.36	0.33	0.35	1.27	1.15	0.90	0.81
9. Marquillo	1.67	0.49	0.29	0.31	0.95	0.78	0.82	0.81
10. Hd. Federation	1.10	0.35	0 32	0.34	1.22	1.15	0.94	0.77
11. Early Triumph	1.10	0.35	0 32	0.31	1.17	1.06	0.91	0.85
12. Axminster	1.76	0.46	0.26	0.30	0.78	0.67	0.86	0.83
13. Renfrew	1.34	0.49	0.37	0.40	1.11	1.01	0.91	0.79
14. Supreme	1.25	0.35	0 28	0.31	1.01	1.01	1.00	0.90
15. Huron	1.76	0.32	0.18	0.15	1.07	0.86	0.80	0.72
16. Kota	1.08	0.33	0.30	0.30	1.10	1.07	0.97	0.74
17. Parker's Sel.	2.00	0.42	0.21	0.18	0.79	0.78	0 99	0 88
Average			0.31	0.32			0 87	0.81

show that they agree quite well. The data of Laboratory C have as means 0.31 and 0.32 for the calculated and observed ratios; Laboratory B gave data with means 0.87 and 0.81. With few exceptions, the differences between ratios obtained in both ways show a range of 5-10%. Evidently the error involved in use of the chromate standard is not unduly large.

Effect of the Different Bleaching Agents

In considering the effect of the different bleachings on these flours it should be borne in mind that the flours were already well aged and presumably had undergone considerable reduction in color before the chemical bleaching agents were applied. No work has been done on the effect of natural aging on the response of flour to chemical bleaching agents and it cannot be said therefore whether the percentage reduction in color noted in this series is greater, equal, or less than would be obtained by treating freshly milled flours.

The best estimate of the color of the unbleached check samples is the average of the determinations made in the three laboratories. The bleached samples, however, were different in each laboratory, as a different bleaching method was used in each. If the colors of the bleached samples were compared with the

average value for the check samples, certain discrepancies would occur because of slight differences in technique. Therefore, in order to use the average color value of the check samples it is necessary to recalculate the value for the bleached samples. This is readily done by use of the ratio bleached to unbleached which was determined for the data of each laboratory. Table II

TABLE II

GASOLINE COLOR VALUES OF UNBLEACHED SAMPLES AND OF SAMPLES BLEACHED IN THREE DIFFERENT WAYS

Variety	Av. gas. color value unbleached	Betachlor, ¾ oz. per bbl.		Novadel, 1 lb. 40 bbl.		Betachlor, ½ oz.per bbl.+Novadel, 1 lb. per 40 bbl.	
		Gas. color value	Ratio B/C	Gas. color value	Ratio B/C	Gas. color value	Ratio B/C
1. Kitchener	1.32	0.83	0.63	0.74	0.56	0.43	0.33
2. Preston	1.18	1.01	0.86	0.59	0.50	0.35	0.30
3. Red Fife	1.17	0.95	0.81	0.51	0.44	0.47	0.40
4. Dicklow	1.15	1.08	0.94	0.58	0.50	0.32	0.28
5. Garnet	1.09	1.00	0.92	0.67	0.62	0.41	0.38
6. Red Bobs	1.11	0.90	0.81	0.56	0.50	0.31	0.28
7. Reward	1.05	0.84	0.80	0.52	0.50	0.41	0.39
8. Marquis	1.06	0.95	0.90	0.53	0.50	0.35	0.33
9. Marquillo	1.24	1.02	0.82	0.67	0.54	0.36	0.29
10. Hd. Federation	1.01	0.95	0.94	0.43	0.43	0.32	0.32
11. Early Triumph	1.02	0.93	0.91	0.39	0.38	0.33	0.32
12. Axminster	1.28	1.10	0.86	0.40	0.31	0.33	0.26
13. Renfrew	1.08	0.98	0.91	0.54	0.50	0.40	0.37
14. Supreme	1.09	1.09	1.00	0.55	0.50	0.30	0.28
15. Huron	1.28	1.02	0.80	0.64	0.50	0.23	0.18
16. Kota	0.99	0.96	0.97	0.38	0.38	0.30	0.30
17. Parker's Selection	1.36	1.35	0.99	0.63	0.46	0.29	0.21
Average			0.87		0.48		0.31

shows the data calculated in this way on the basis of the mean of the check samples. The average reduction in color effected by the three bleaches was: Betachlor, 13%; Novadel, 52%; Betachlor followed by Novadel, 69%. It is of interest to note that with the combination of Betachlor and Novadel the final color values were very similar, indicating that generally the more highly pigmented flours react to a relatively greater extent than the less highly pigmented flours. Thus the very yellow Parker's Selection with an original color value of 1.36 bleaches down to 0.21, a reduction of 79%, while the light colored Kota of color 0.99 is reduced in color 70% to a value of 0.30. A large reduction of intensity is found in all the highly pigmented flours, namely, Kitchener, Marquillo, Axminster, and Huron, and confirms an observation previously made by one of us* that in the flour of Marquillo-Marquis crosses, those samples carrying heavy color are bleached to a greater degree than the less heavily colored samples in the same treatment. This is a very interesting observation and one that if conclusively proved would have an important bearing on the

**Private communication.*

problems of plant breeders engaged in the production of improved varieties of wheat. In the past considerable emphasis has been laid on color and doubtless selections which were otherwise promising have been discarded on this account. In testing new strains and selections it would be advisable, therefore, to study the reaction to bleaching agents rather than only the color of the unbleached sample.

The results so far considered show that by use of either Novadel, or a combination of Betachlor and Novadel, the yellow color of the flour of any of the varieties studied can be satisfactorily reduced. It frequently occurs, however, that bleaching which is too severe has a bad effect on the bread color, producing, in place of the objectionable yellow tint, a more objectionable grey, or in some cases, a pale pasty color. An examination of the crumb color scores assigned in the various bakings should give an estimate of whether or not such changes had occurred as a result of the bleaching.

It is deplorable that the scoring of bread color still remains a matter of personal judgment of the baker, as no adequate apparatus or procedure for obtaining a more accurate estimate has been developed. However, it may be said that an experienced baker in any given laboratory will have a nicely adjusted scale of color values that may be relied upon to give fairly accurate relative results for scores made in that laboratory. The three collaborating laboratories have attempted to standardize color scoring of bread by using a common standard flour, and by comparing from time to time scores made on different samples of bread. The color scores are designated by numbers as follows:— 8, creamy yellow or dull white; 7, yellow; 6, dull yellow or very slightly grey; 5, very yellow or slightly grey; 4, greyish yellow; 3, dull grey; 2, very grey; 1, greyish brown. "Creamy yellow" is considered two points better than "very slightly grey", while "very yellow" is three points better than "very grey". Therefore, if an unbleached flour gave yellow bread, any hint of grey in the bread from bleached flour would result in a reduction of color score. With these points in mind, the bread color scores found with four baking formulas may be examined to see how the changes effected by bleaching the flour are manifested in the baking results.

Considering first the flours bleached with Betachlor only, the results in Table III indicate that no appreciable change in bread color occurred. The improvement in bread color by Novadel and Betachlor-Novadel bleaching is shown in Tables IV and V by the actual and percentage increases in color scores. Novadel bleaching (Table IV) gave an increase of one or more points in all samples except 10 and 14 (0.001% bromate baking). In these data there is no suggestion whatever of damage to color, but on the contrary every evidence of marked improvement. The samples bleached with Betachlor plus Novadel showed generally a greater improvement in bread color than the Novadel bleached samples. It can be seen from Table V that there was in no case, by any of the baking formulas applied, any decrease but rather a very distinct improvement in crumb color. It appears evident that all these varieties can be bleached satisfactorily from the standpoint of both flour color

and bread color and it is doubtful, therefore, that they can be differentiated by this means.

TABLE III

Improvement in crumb color of the bread due to bleaching with Betachlor only. Expressed in actual points and in percentages

Variety	Improvement in crumb color of bleached samples							
	Simple		Bromate (0.001%)		Blend-bromate		Diastatic malt and phosphate	
	Points	%	Points	%	Points	%	Points	%
1. Kitchener	1.5	25	0	0	1.0	14	1.0	14
2. Preston	0.5	8	0	0	0.5	7	0.5	7
3. Red Fife	1.0	17	0	0	0.1	14	0.5	7
4. Dicklow	1.5	25	0	0	0.5	7	0.5	7
5. Garnet	0.0	0	0.5	7	0.5	7	0.0	0
6. Red Bobs	1.0	17	0.5	6	0.0	0	1.0	14
7. Reward	1.0	17	0.0	0	0.0	0	1.0	14
8. Marquis	0.5	8	0.0	0	0.0	0	−0.5	−7
9. Marquillo	0.0	0	−0.5	−6	0.0	0	−0.5	−8
10. Hd. Federation	0.0	0	0.5	6	0.0	0	0.5	7
11. Early Triumph	0.0	0	0.5	6	0.0	0	0.0	0
12. Axminster	0.5	8	0.5	7	0.5	7	0.5	7
13. Renfrew	0.0	0	0.5	7	0.0	0	0.0	0
14. Supreme	0.0	0	0.0	0	0.0	0	0.5	7
15. Huron	−0.5	−8	0.0	0	0.0	0	0.0	0
16. Kota	0.0	0	0.0	0	0.0	0	−0.5	−7
17. Parker's Sel.	0.0	0	0.0	0	−0.5	−6	0.0	0

TABLE IV

Improvement in crumb color of the bread due to bleaching with Novadel. Expressed in actual points and in percentages

Variety	Improvement in crumb color of bleached samples											
	Simple		Bromate (0.001%)		Bromate (0.002%)		Blend-bromate		Malt-arkady		Diamalt & phosphate	
	Points	%	Points	%	Points	%	Points	%	Points	%	Points	%
1. Kitchener	2	33	2	33	2	33	2	29	1.5	25	2	33
2. Preston	2	33	2	33	3	50	1	14	1.5	25	2	33
3. Red Fife	2	33	1.5	20	2	33	2	29	1.5	25	2	33
4. Dicklow	2	33	1	14	1	14	1.5	20	2	33	2	33
5. Garnet	1	17	1	14	2	33	3	50	1	18	1	17
6. Red Bobs	1	14	1.5	20	3	50	2	29	1	15	1	14
7. Reward	2	29	1.5	20	2	29	1.5	20	2	31	2	29
8. Marquis	2	33	1.5	20	1	14	2	29	2	36	2	33
9. Marquillo	2	40	1.5	25	3	60	1.5	25	2	40	2	40
10. Hd. Federation	2	29	0.5	7	3	50	2	29	2	31	2	29
11. Early Triumph	3	50	2	29	3	50	2	29	3	54	3	50
12. Axminster	2.5	45	1	17	3	60	3	50	1.5	50	2.5	45
13. Renfrew	1	14	1.5	20	1	14	2	29	1.5	25	1	14
14. Supreme	3.5	64	0.5	7	1	14	2	29	3.5	70	3.5	64
15. Huron	2.5	45	2	33	2	29	2	29	2.5	50	2.5	45
16. Kota	2.5	45	1	14	2	33	2	29	2.5	50	2.5	45
17. Parker's Sel.	2	40	1.5	50	3	60	1.5	25	2	40	1.5	27

TABLE V

IMPROVEMENT IN CRUMB COLOR OF THE BREAD DUE TO BLEACHING WITH BETACHLOR FOLLOWED BY NOVADEL. EXPRESSED IN ACTUAL POINTS AND IN PERCENTAGES

Variety	Improvement in crumb color of bleached samples											
	Simple		Bromate (0.001%)		Bromate (0.002%)		Blend-bromate		Malt-arkady		Diamalt & phosphate	
	Points	%	Points	%	Points	%	Points	%	Points	%	Points	%
1. Kitchener	3	50	1.5	20	2	27	1.5	20	2	29	2.5	38
2. Preston	4	80	3	46	2	29	2.5	36	1.5	21	3	54
3. Red Fife	4	67	2	27	1.5	18	1.5	18	2.5	33	2	29
4. Dicklow	2	29	1.5	19	1	12	0.5	6	1	14	2	29
5. Garnet	3	50	1.5	20	2	29	1	12	0.5	9	2	33
6. Red Bobs	3.5	54	2.5	33	2	25	1	11	1.5	19	2	29
7. Reward	1.5	20	1.5	18	2	25	1.5	18	2	33	2	29
8. Marquis	2.5	36	2	25	3	40	1	11	2	29	1.5	21
9. Marquillo	3.5	78	2.5	6	2.5	36	2.5	36	3	50	3	60
10. Hd. Federation	2	29	2	27	2	25	2	25	2	29	2	29
11. Early Triumph	2	31	2	25	2.5	33	2	25	2	29	3	50
12. Axminster	3.5	70	2.5	36	3.5	58	3	43	3	60	4	80
13. Renfrew	1.5	21	1.5	17	2	25	1	11	2	29	2	29
14. Supreme	2.5	42	2	25	3	43	1.5	18	2	29	2	29
15. Huron	2	40	2.5	36	2.5	36	1.5	19	3	50	2	17
16. Kota	1.5	27	2	29	2.5	33	1.5	18	2	29	1.5	27
17. Parker's Sel.	2.5	50	3.5	64	2.5	38	2.5	38	3	60	1.5	30

Effect of Bleaching on Loaf Volume

Bleaching agents that contain chlorine have, in addition to their effect on color, an aging effect on flour. An overdosage of the bleaching agent will injure the baking quality. This type of damage is known as "overbleaching". Larmour and Machon (7) have shown that this effect is related to protein content and loaf volume of the flour, and that flours very low in protein are more readily overbleached than the average of high protein flours. Overbleaching can usually be detected most readily by use of formulas involving oxidizing agents because, if the bleaching has modified the flour to its limit, further action of powerful oxidizers will cause it to break in strength, thus producing lower loaf volumes than are obtained when the unbleached flour is baked in the same way. Thus the simple and malt-phosphate formulas are useful in estimating the extent to which maturation has progressed, while the bromate, blend-bromate, and malt-arkady formulas will expose any weakness occasioned by carrying the maturation process too close to the limit of strength of the flours. Malloch, Geddes, and Larmour (8) have shown that in these varieties there are real differences in strength, as judged by the baking data. The authors were interested in ascertaining if their behavior after bleaching would reveal further qualitative differences.

For convenience in tabulating the data, the loaf volume of the bleached sample has been calculated as percentage of the corresponding unbleached samples and for each variety the averages of the simple and malt-phosphate

data, and of the data from formulas involving an oxidizer have been computed. The results are given in Tables VI, VII, and VIII.

TABLE VI

Loaf volume of bleached samples as percentage of loaf volume of check samples (Betachlor bleach)

Variety	Simple	Bromate (0.001%)	Blend-bromate	Diastatic malt and phosphate	Mean simple and malt phosphate	Mean bromate and blend-bromate
1. Kitchener	105	92	92	112	108.5	92
2. Preston	107	97	101	106	106.5	99
3. Red Fife	105	93	95	104	104.5	94
4. Dicklow	101	104	96	110	105.5	100
5. Garnet	99	92	102	102	100.5	97
6. Red Bobs	104	99	101	102	103	100
7. Reward	108	98	105	106	107	101
8. Marquis	102	107	97	100	101	102
9. Marquillo	104	111	102	100	102	106.5
10. Hd. Federation	102	104	103	102	102	103.5
11. Early Triumph	99	102	103	103	101	102.5
12. Axminster	97	104	101	98	97.5	102.5
13. Renfrew	101	97	105	102	101.5	101
14. Supreme	99	112	108	97	98	110
15. Huron	104	91	104	99	101.5	97.5
16. Kota	102	104	99	104	103	101.5
17. Parker's Sel.	97	98	128	94	95.5	113.5
Mean	102	100.3	102.7	102.4		

TABLE VII

Loaf volume of bleached as percentage of loaf volume of check samples (Novadel bleach)

Variety	Simple	Bromate (0.001%)	Bromate (0.002%)	Blend-bromate	Malt-arkady	Diastatic malt and phosphate	Mean
1. Kitchener	102	101	101	97	101	102	0.5
2. Preston	102	98	101	101	106	97	1.0
3. Red Fife	99	100	104	97	101	101	0
4. Dicklow	102	99	98	101	101	106	1
5. Garnet	103	106	102	100	99	100	1.5
6. Red Bobs	101	94	97	97	96	98	−3
7. Reward	102	103	101	100	101	103	1.5
8. Marquis	102	99	100	102	99	98	0
9. Marquillo	100	98	101	99	96	98	−1
10. Hd. Federation	100	101	97	97	103	101	0
11. Early Triumph	103	102	99	98	97	100	0
12. Axminster	98	95	99	98	103	94	−2
13. Renfrew	98	103	103	100	101	100	1
14. Supreme	99	101	101	98	102	103	0.5
15. Huron	98	101	101	100	102	101	0.5
16. Kota	100	101	101	102	101	98	0.5
17. Parker's Sel.	101	99	102	102	98	100	0.5
Mean	100.6	100	100.5	99.4	100.4	100	

TABLE VIII

Loaf volume of bleached as percentage of check samples (Betachlor plus Novadel bleach)

Variety	Simple	Bromate (0.001%)	Bromate (0.002%)	Blend-bromate	Malt-arkady	Malt-phos-phate	Mean, simple and malt-phosphate	Mean of oxidizer[1] formulas
1. Kitchener	104	96	96	101	100	104	104	98.2
2. Preston	110	94	98	102	99	109	109.5	98.2
3. Red Fife	105	100	95	98	103	106	105.5	98.8
4. Dicklow	102	106	101	95	94	100	101	97.5
5. Garnet	102	98	98	102	102	107	104.5	100
6. Red Bobs	105	97	100	100	101	106	105.5	99.5
7. Reward	105	93	95	99	101	105	105	97
8. Marquis	104	89	95	104	96	105	104.5	96
9. Marquillo	106	98	101	98	104	104	105	100.2
10. Hd. Federation	105	95	93	100	98	103	104	96.5
11. Early Triumph	106	98	93	100	97	104	105	97
12. Axminster	104	94	95	103	98	103	103.5	97.5
13. Renfrew	106	95	96	99	94	101	103.5	96
14. Supreme	102	100	97	97	96	103	102.5	97
15. Huron	101	98	99	101	95	109	105	98.2
16. Kota	100	102	97	96	97	103	101.5	98
17. Parker's Sel.	103	99	99	103	98	105	104	99.8
Mean	104	97	97	100	98	104.5		

The data with the Betachlor bleach, in Table VI, show that some maturing occurred in all varieties except Axminster, Supreme and Parker's Selection. These three gave loaf volumes slightly below the checks by the simple and malt-phosphate formulas, but as they all showed an increase by the bromate formulas the differences noted cannot be considered significant. When baked by the bromate formulas, Kitchener, Red Fife, Garnet, and Huron gave a decrease which is probably significant only in the first two. On the whole it may be concluded that the Betachlor bleach improved the loaf volume without causing any weakness except possibly in Kitchener and Red Fife.

Novadel alone is reputed to have little or no effect in respect to maturing and it is not surprising, therefore, that the data in Table VII show no differences that may be considered significant.

Table VIII shows that in practically all cases the double bleaching with Betachlor plus Novadel resulted in increased loaf volume when the samples were baked by the simple and malt-phosphate formulas. With the bromate formulas there was generally a slight, perhaps non-significant, decrease in loaf volume. The greatest decrease noted was 11% with Marquis in the 0.001% bromate baking. The varieties showing the greatest decreases in the average of the four bromate formulas were: Marquis and Renfrew each, 4%; Hard Federation, 3.5%; Reward, Early Triumph and Supreme each, 3%.

This examination of the loaf volume data indicates that all these varieties were satisfactorily bleached, that no appreciable overbleaching occurred, and that the chlorine bleaches produced a slight degree of maturing

despite the fact that the flours were all quite well aged before bleaching. Application of normal dosages of these two bleaching agents alone or in combination produced no depreciation of quality in any of the 17 varieties studied and this method, therefore, provided no means for differentiating them in respect to strength. In order to serve this purpose it is likely that abnormal dosages would have to be applied as in the study made by Larmour and Machon (7). It would be necessary to select a dosage of a chlorine bleach that would mature an average or standard variety to its limit and observe what varieties were damaged by this treatment. This would be of scientific interest but of no practical significance as a test method, because more rapidly applied tests of strength are available.

References

1. Assoc. of Official Agricultural Chemists. Official and Tentative Methods of Analysis. 3rd ed. Washington. 1930.
2. Coleman, D. A. and Christie, A. Cereal Chem. 3: 84-89. 1926.
3. Ferrari, C. G. and Bailey, C. H. Cereal Chem. 6: 218-240. 1929.
4. Jorgensen, H. Cereal Chem. 4: 468-469. 1927.
5. Kent-Jones, D. W. and Herd, C. W. Cereal Chem. 6: 33-50. 1929.
6. Larmour, R. K. and Brockington, S. F. Can. J. Research, 5: 491-500. 1931.
7. Larmour, R. K. and Machon, F. D. Can. J. Research, 4: 399-420. 1931.
8. Malloch, J. G., Geddes, W. F. and Larmour, R. K. Can. J. Research. In preparation.
9. Visser't Hooft, F. and de Leeuw, F. J. G. Cereal Chem. 5: 351-365. 1928.

THE KINETICS OF THE OXIDATION OF GASEOUS ACETONE[1]

By E. W. R. Steacie[2]

Abstract

The oxidation of gaseous acetone is a homogeneous chain reaction between 350° and 500° C. The effect of pressure on the rate of the reaction indicates an "order" somewhat greater than three. The indications are that the first step in the reaction consists of the formation of an unstable peroxide. The predominant reaction then appears to be the formation of acetic and formic acids together with their products of oxidation and decomposition. The actual course of the reaction varies somewhat as the temperature changes.

The temperature coefficient and the effects of surface and of foreign gases show that the chain length is comparatively short and varies with temperature. The process by which the chains are initiated is probably bimolecular. The reaction differs from most oxidation reactions of the chain type in that the concentrations of the two reactants are about equally important in so far as their effect on the rate of the reaction is concerned.

Introduction

The idea of reaction chains was originally put forward to explain those photochemical reactions in which more than one molecule reacts per quantum of light absorbed. Recently, however, it has been shown that certain thermal reactions may occur by a chain mechanism. In such cases the first step is a reaction of an ordinary bimolecular or termolecular type. This gives rise to a product molecule endowed with excess energy (the heat of activation plus the heat of reaction). This activated product molecule then activates a fresh molecule of the reactant by collision, and so on, a reaction chain being produced. This reaction chain is finally broken by the deactivation of a molecule either in the gas or at the wall of the containing vessel.

It is obvious that a mechanism of this type will be most likely in the case of a highly exothermic reaction. Gaseous oxidation reactions are therefore suitable for investigation.

Hinshelwood and his coworkers have investigated the oxidation of hydrogen. (8, 9, 10, 13), ethylene (14), benzene (3), methane, methyl alcohol, and formaldehyde (4). All these reactions occur by a chain mechanism. Those which involve the oxidation of organic compounds have certain points in common: (1) The reactions possess no simple integral order. The apparent order is high (usually between three and four) and variable. (2) The rate is greatly dependent on the concentration of the organic substance, but is little influenced by the oxygen concentration. (3) Increase in the surface of the containing vessel retards the reactions. (4) The rate of change of pressure accompanying the oxidation attains its full value only after an induction period. Hinshelwood accepts the suggestion of Egerton (2) that the induction periods are due to the initial formation of unstable peroxides which serve as centres from which the chains are propagated.

[1] *Manuscript received January 16, 1932.*
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It is of interest to accumulate more data concerning such reactions. In addition *it is highly desirable to investigate the oxidation of simple organic* substances under definite conditions, such as those which prevail in the gaseous state. The present paper deals with the gas-phase oxidation of acetone.

Apparatus

The reaction was investigated by introducing oxygen-acetone mixtures into a heated vessel and observing the rate of change of pressure as the reaction proceeded.

The apparatus employed is illustrated in Fig. 1. The reaction bulb, *A*, of Pyrex glass, was contained in the electric furnace, *B*. The furnace was of a type which has been previously described (12). Temperatures were measured by means of the chromel-alumel thermocouple, *D*, in conjunction with a Cambridge workshop pattern potentiometer. The temperature was hand regulated by means of a rheostat, and could be kept constant to within 1° C.

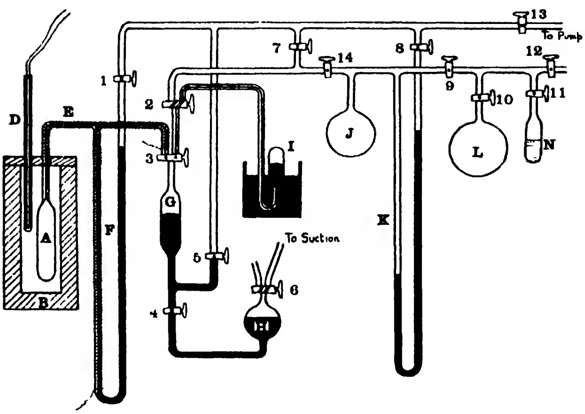

Fig. 1. *Diagram of apparatus.*

The reaction bulb was connected through the Pyrex to soft glass seal, *E*, with the capillary manometer, *F*. This manometer and all connecting tubing up to within two or three centimetres of tap 3 was wound with nichrome wire, and maintained at a temperature of 100° C.

Oxygen and acetone were stored in the containers, *L* and *N*. Mixtures of the desired proportions were made up by admitting the gases to the reservoir, *J*, and observing their partial pressures by means of the manometer, *K*.

The oxygen employed was obtained from cylinders, and was purified only by drying over phosphorus pentoxide. It contained about 0.5% of nitrogen.

Acetone was purified by distillation from alkaline permanganate, followed by fractional distillation. It was freed from dissolved air by repeated evacuation.

Experimental Procedure

Prior to making an experiment, suction was applied to bulb *H*, through tap 6. Taps 4 and 5 were opened, and the mercury in the bulb, *G*, and the connecting tubing up to tap 5, was sucked into *H*. Tap 4 was then closed. By suitable adjustment of tap 3, it was then possible to connect the reaction vessel, *A*, to the pumping system for evacuation. The pumping system consisted of a low and a high stage mercury condensation pump in series, backed up by a Hyvac oil pump. Pressures during the process of evacuation were measured by means of a McLeod gauge.

When the system had been thoroughly evacuated, the furnace was brought to the desired temperature as indicated by the thermocouple, *D*. A sample of the oxygen-acetone mixture from the bulb, *J*, was then introduced into the reaction bulb as follows:

Tap 5 was closed and tap 6 was turned so as to connect the bulb, *H*, to the atmosphere. On opening tap 4, mercury entered the bulb *G* from *H*, and was allowed to rise until it had reached some pre-determined level in the bulb *G*, such as the position indicated in the diagram. Tap 7 was then closed, and taps 3, 2, and 14 were turned so as to allow some of the mixture in the reservoir, *J*, to enter the bulb *G*. Tap 3 was then turned to connect *G* with *A*. Tap 4 was opened, and mercury was allowed to follow up the gas, pass through tap 3, and rise about 10 cm. in the capillary above tap 3. In this way the entire space occupied by the reacting gases could be kept hot to prevent the condensation of products, without the necessity of employing a troublesome heated stopcock. The possibility of complications due to the contact of acetone with stopcock grease was also eliminated.

As soon as the reactants had been admitted to *A*, the pressure was read on the capillary manometer, *F*. Pressure readings were then taken at regular intervals of time, and the progress of the reaction was followed by the rate of increase in pressure. The correction for the capillary depression in the manometer, *F*, was made automatically by taking the zero reading of the manometer as the position of the mercury when the system was evacuated. Since relative pressures only are important, no correction was made for the temperature of the mercury in the manometer, and all pressures are expressed in terms of cm. of mercury at 100° C.

The volume of the "dead space" outside the furnace was only about 1% of the volume of the reaction vessel. In consequence it could be neglected without serious error.

In order to withdraw a sample of gas for analysis on the completion of a run, tap 3 was turned so as to connect the reaction vessel to *G*. Tap 4 was opened, suction was applied to *H*, and a sample of the products of reaction was sucked into *G*. Taps 3 and 2 were then adjusted, air pressure was applied to the bulb *H*, and the gaseous sample was forced into the tube, *I*, and collected over mercury.

The Products of the Reaction

The gaseous products of the reaction were analyzed in a modified type of Bone-Newitt gas analysis apparatus.

The condensable products could be estimated, and the gaseous products referred back to unit quantities of reactants in two ways. First by mixing a known amount of nitrogen or some other inert gas with the original reactants, and calculating back from the amount of nitrogen found in the gaseous products. Secondly by the following method: An inert gas was introduced into the reaction vessel under the conditions which would normally be used in an experiment. The pressure in *A* was noted. A sample was then withdrawn and collected in *I* in the usual manner. The decrease in pressure produced in *A* by the

removal of the sample was noted. The gas in *I* was then transferred completely to the gas analysis apparatus and its volume was measured. In this way the apparatus was calibrated so that the pressure drop which accompanied the removal of a sample indicated its total volume expressed at room temperature and atmospheric pressure. The actual volume of any sample was measured, and the condensable products were determined by difference. Before the measurement, unchanged acetone, or acetic acid, was removed from the sample by absorption with distilled water (15).

The oxidation of acetone in the liquid state, or in solution, has been widely investigated. Very little previous work has been done, however, on the oxidation of gaseous acetone.

White and Price (16) have investigated the explosion limits of acetone-air mixtures, and Holm (11) has measured ignition temperatures. No data are given in these papers concerning the products of the oxidation. Wheeler and Whitaker (15) also investigated oxygen-acetone explosions. They state that on explosion complete oxidation occurs, as indicated by the equation

$$CH_3COCH_3+4\ O_2=3\ CO_2+3\ H_2O.$$

The conditions during explosion are so drastic, however, that this information is of little use in predicting what will occur during the slow oxidation of acetone.

The only direct investigation of the oxidation which the writer has been able to discover is that of Gottlieb (5). He states that the main reaction on passing oxygen-acetone mixtures over a hot surface is the formation of formic and acetic acids,

$$CH_3COCH_3+1.5\ O_2=CH_3COOH+HCOOH.$$

This mechanism receives support from the general chemical behavior of acetone on oxidation under other conditions. The work of Gottlieb is somewhat open to suspicion, however, since it was done in the early days of the development of organic chemistry (1844).

It has been shown by Bone that under the conditions which prevailed in the present investigation formic acid will decompose much more rapidly than it oxidizes (1, pp. 358-400). Hence if Gottlieb's work is correct, we should expect the reaction to be represented by

$$CH_3COCH_3+1.5\ O_2=CH_3COOH+H_2O+CO.$$

This would lead to an increase in pressure equal to 50% of the initial concentration of acetone. The actual pressure increase is much higher than this (120 to 150%), the oxygen consumption is greater than that indicated above, and the amounts of carbon monoxide and of condensable products formed are also greater.

It may therefore be concluded that the above reaction is also accompanied by a considerable amount of direct oxidation of acetone to carbon monoxide and water, *viz.*,

$$CH_3COCH_3+2.5\ O_2=3CO+3\ H_2O.$$

A combination of these two modes of oxidation in roughly equal proportions would lead to the pressure increases at completion and to the products found.

A certain amount of carbon dioxide is also found. This may arise by oxidation of carbon monoxide, or, more probably, by a small amount of oxidation of formic acid rather than its decomposition.

It may be pointed out that formaldehyde and methyl alcohol cannot be postulated as products of the oxidation of acetone, since Fort and Hinshelwood (4) have shown that they oxidize at a much faster rate than has been found here for acetone.

For our purpose, however, the important point is not the exact mechanism of the oxidation, but rather to make certain that the pressure increases obtained under different conditions of temperature and concentration are comparable and furnish an indication of the rate at which acetone and oxygen disappear from the system. We will therefore examine the results of the analyses of the products of the reaction at different temperatures, and for different relative proportions of the two reactants.

(A) The Effect of Temperature on the Products of Reaction

In Table I are tabulated the average results of a number of analyses of the products formed by the reaction of a mixture containing 1 mole of acetone to 4.05 moles of oxygen at various temperatures. The analyses are given for completion, and also for a pressure increase equal to 50% of the original partial pressure of acetone. These are corrected for the small amount of nitrogen present in the original mixture.

TABLE I

ANALYSIS OF PRODUCTS FROM 1 $CH_3COCH_3+4.05\ O_2$

Temperature °C.	Stage	O_2 %	CO_2 %	CO %	Condensable (including unchanged acetone), %	CH_4 %
350	Completion	33.0	6.3	16.1	43.6	1.0
	50%	62.0	1.7	5.1	33.8	0.0
400	Completion	30.0	7.6	18.9	43.4	0.1
	50%	59.9	0.9	7.7	29.5	2.0
450	Completion	32.7	7.5	17.5	42.4	0.0
	50%	60.8	1.6	7.4	28.6	1.6
500	Completion	17.6	9.5	12.5	60.3	0.2
	50%	59.8	1.3	7.6	29.0	2.3

In addition, tests were carried out on the condensable products for acetic acid, and for formaldehyde. Acetic acid was definitely shown to be present in all cases by means of the cacodyl oxide test. No trace of formaldehyde could be detected by means of Schryver's test.

It will be seen from Table I that there is satisfactory correspondence in the analyses at all temperatures at the 50% pressure increase stage. At completion there is also good agreement at 350°, 400°, and 450° C. At 500° C.,

the oxygen consumption is much greater and more condensable products are formed. This is in agreement with the fact that there is a greater total increase in pressure at completion at 500° C., as will be shown later.

It would therefore seem to be justifiable to compare results in the earlier stages of the reaction at all temperatures.

B. The Effect of the Relative Proportions of the Reactants

In Table II are tabulated the analyses of the products of reaction for different mixtures at 450° C. In order to make these comparable they are expressed as per cent of constituents other than oxygen.

TABLE II

ANALYSIS OF PRODUCTS FROM DIFFERENT MIXTURES AT 450° C.

O_2/acetone	Stage	CO_2 %	CO %	Condensable, %	CH_4 %	$CO+CO_2$, %	CO/CO_2
4.05	Completion	11.1	26.0	62.9	0 0	37.1	2 3
	50%	4.0	18.8	73.2	4 0	22.8	4.7
2.98	Completion	7.8	28.1	62.7	1.4	35.9	3.6
	50%	4 3	18.6	76.6	0 5	22.9	4.3
2.16	Completion	2.6	35.5	60.1	1.8	38.1	13.7
	50%	4.1	20.7	73.2	2.0	24.8	5.0

The results in Table II show that there is again excellent correspondence in analyses at the 50% stage. At completion there is also fair correspondence, except that, as might be expected, the CO/CO_2 ratio increases as the amount of oxygen in the mixture decreases.

The Pressure Change Accompanying the Reaction

As pointed out above, oxidation to acetic acid and the decomposition products of formic acid would result in a pressure increase equal to 50% of the initial partial pressure of the acetone. Direct oxidation to carbon monoxide and water, on the other hand, would lead to a pressure increase equal to 250% of the initial acetone pressure. The pressure increases actually obtained are given in Table III. These support the conclusion that the actual reaction is a combination of the two possibilities mentioned above. The "final" pressure increases listed were taken when the pressure had remained constant for from 4 to 12 hr. On very long standing (2 to 10 days), especially at the higher temperatures, small erratic pressure changes occurred depending presumably on the slow secondary oxidation of some of the products.

The pressure increase is therefore in the neighborhood of 125%, becoming somewhat higher at high temperatures. This is in accordance with the analytical results, since the products of the reaction change their character somewhat at higher temperatures.

TABLE III

PRESSURE INCREASES AT COMPLETION

Partial acetone pressure, cm.	O_2/acetone	Increase in pressure, as % of initial partial pressure of acetone	Partial acetone pressure, cm.	O_2/acetone	Increase in pressure, as % of initial partial pressure of acetone
Temperature, 350° C.			*Temperature, 400° C.*		
4.0	4.95	117 8	9 7	3.10	125 0
7.45	4.95	127.6	9.6	3.65	120 5
7.5	3 03	120 4	3.5	4.35	123 2
9.7	3.03	121.2	7 3	5 00	123 0
16.0	2.00	117.0	8.7	3.00*	123.8
			5 0	3 00†	113.6
Temperature, 450° C.			*Temperature, 500° C.*		
14.2	2.98	128 0	11 9	2 16	142 6
8.2	4.60	138 6	7.65	2.16	145.9
10.8	4 05	141.1	2 65	2 16	151 0
12.8	2 16	127 5			
13.5	2 16	135 0			

*Plus N_2. †*Plus tubing in reaction vessel.*

The Velocity of the Reaction

From the previous sections it may be concluded that the rate of pressure change furnishes a sufficiently accurate indication of the rate at which acetone is oxidized. We will therefore consider the velocity of the reaction as inferred from pressure-time curves. The effect of individual factors, such as pressure, concentrations, and temperature, will be considered in detail later.

Some typical data are summarized in Table IV.

The effect of the total pressure on the rate of the reaction is illustrated in Fig. 2, in which pressure increase-time curves are given for the same mixture and temperature, but for varying initial pressures.

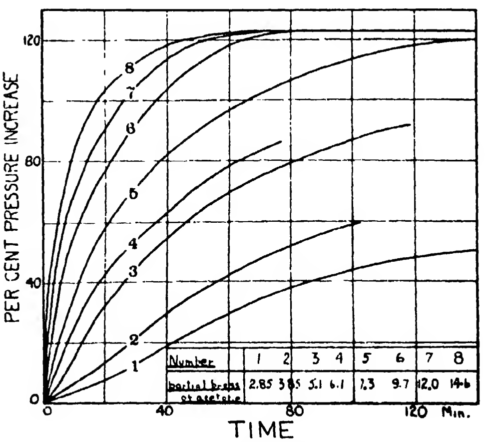

FIG. 2. *The effect of pressure on the rate of reaction; 400°C.* 1 CH_3COCH_3+3.1 O_2.

The Form of the Reaction Velocity Curves

It will be seen from Fig. 2, and Table IV that there is an induction period at the beginning of the reaction. The maximum rate of change is not reached until a certain time has elapsed. On account of the form of the curves it is impossible to obtain a simple mathematical expression for them. Hinshelwood has successfully derived an equation for such a reaction (4), but this is very complicated and contains a

number of constants which must be evaluated experimentally. The simplest method of ascertaining the effect of various factors on the rate of reaction is therefore to compare the times for some arbitrarily defined fractional change, such as the time to half-value.

TABLE IV

TYPICAL REACTION VELOCITY DATA

Time, min.	Pressure, cm.	Pressure, increase, cm.	Increase, % of initial partial pressure of acetone	Time, min.	Pressure, cm.	Pressure increase, cm.	Increase, % of initial partial pressure of acetone
400° C. 1 CH_3COCH_3+5.00 O_2							
0.0	56.9	0.0	0.0	5	61.65	4.75	51.1
0.5	57.8	0.9	9.7	7	62.65	5.75	61.9
1	58.4	1.5	16.2	11	64.05	7.15	77.0
2	59.4	2.5	26.8	15	65.0	8.1	87.0
3	60.3	3.4	36.5	40	67.45	10.55	113.5
4	61.05	4.15	44.6	Completion	68.2	11.3	121 5
400° C. 1 CH_3COCH_3+2.03 O_2							
0	24.9	0.0	0.0	32.5	30.3	5.4	65.8
1	25.15	0.25	3.0	41.5	30.9	6.0	73.2
3	25.35	0.45	5.5	52	31.45	6.55	79.9
7	26 35	1.45	17.7	63.5	31.95	7.05	86.0
11	27.4	2.5	30.5	74	32.3	7.4	90.0
15	28 2	3.3	40.2	Completion	34 7	9.8	119.4
21	29.15	4.25	51.8				
500° C. 1 CH_3COCH_3+2.16 O_2							
0.0	29.1	0.0	0.0	3	41.1	12.0	130.5
0.25	30 7	1.6	17.4	4	41.35	12.25	133.0
0.5	33.0	3.9	42.4	6	41.8	12.7	138.0
0.75	35.4	6.3	68.5	8	42.0	12.9	140.1
1.0	36.9	7.8	84.7	10	42.05	12.95	140.8
1.5	39.0	9.9	107.5	20	42.45	13.35	145.0
2	40.15	11.05	120.0	1200	42.45	13.35	145.0

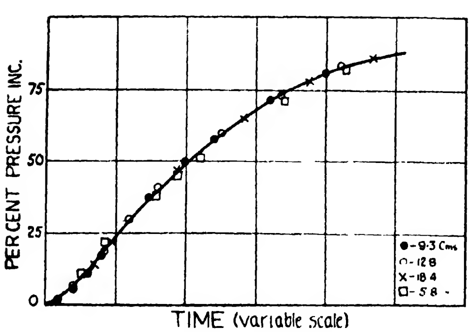

FIG. 3. *Affine curves. Varying pressure. 400°C.* 1 CH_3COCH_3+2.0 O_2. *The pressures given above are the initial partial pressures of acetone.*

If such a criterion is used, however, it is necessary to make sure that the form of the curves does not alter appreciably when the various factors affecting the rate are altered. This can be most simply done by determining whether the various curves are "affine". To do this, a set of curves is first plotted in the ordinary way. One curve is then adopted as the standard, and the time scale of the others is altered so as to make one arbitrarily chosen point coincide for all the curves. If the curves

are affine, there will then be complete correspondence throughout their entire length.

Fig. 3 shows curves plotted in this way for a 1 : 2.0 acetone-oxygen mixture at 450° C., with varying initial pressures. It will be seen that the curves are identical in form throughout their entire length. The relative importance of the induction period is evidently the same for all pressures.

Fig. 4 shows similar curves in which the temperature and the initial acetone pressure are kept constant, while the amount of oxygen varies from 1 to 2, to 1 to 4.6. There is again complete correspondence of the curves at every stage. It would therefore appear that at any particular temperature we are justified in adopting the time for any desired fractional change as a criterion of the rate of the reaction.

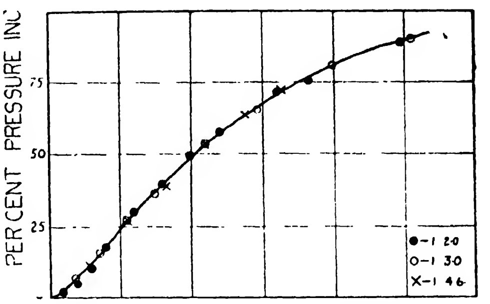

FIG. 4. *Affine curves with varying oxygen-acetone ratios. 450° C. Partial acetone pressure, 9.6 cm.*

The effect of varying temperature on the form of the curve is illustrated in Fig. 5. It is apparent that the strict correspondence of the curves, as shown in Figs. 3 and 4, no longer exists. The form of the curves varies with temperature, the induction period being relatively more pronounced at higher temperatures. The correspondence of the curves at 500°, 450° and 400° C. is good enough in the early stages to allow a sufficiently accurate comparison to be made by means of the time to some fractional change. Such a comparison, however, is not warranted for the curve at 350° C. In order to eliminate the effect of the induction period, and thus allow at least a rough comparison of the curve at 350° C. with the others, the time for the reaction to proceed from 30 to 50% has been used. Any conclusions drawn from such values will be completely justified for any particular temperature. At different temperatures the results will be sufficiently accurate for all but the curve at 350° C. and merely a rough approximation for this temperature.

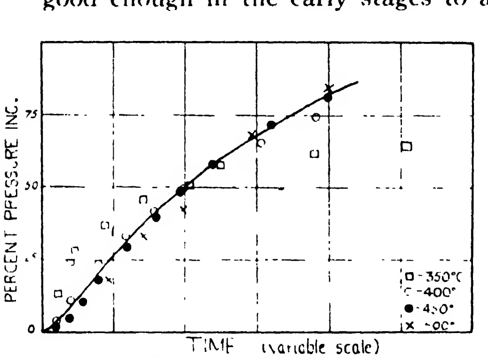

FIG. 5. *Affine curves with varying temperatures. Partial acetone pressure, 9.6 cm.* 1 $CH_3COCH_3+2.0\ O_2$.

The Effect of the Total Pressure on the Rate of the Reaction

The times for the reaction to proceed from 30 to 50% pressure increase under various conditions are given in Tables V, VI, VII and VIII.

TABLE V

VALUES OF $T_{60}-T_{30}$ AT 350° C.

O_2/acetone = 2.00		O_2/acetone = 3.03		O_2/acetone = 4.95	
Partial acetone pressure, cm.	$T_{60}-T_{30}$, min.	Partial acetone pressure, cm.	$T_{60}-T_{30}$, min.	Partial acetone pressure, cm.	$T_{60}-T_{30}$, min.
16.0	5.3	9.7	11	7.4	17
14.9	7.4	7.5	22	7.25	17
11.4	21	5.1	65	4.05	52
9.6	36	4.6	87	1.75	Large
7.5	144	2.3	Large		

TABLE VI

VALUES OF $T_{60}-T_{30}$ AT 400° C.

	O_2/acetone = 2.03			
Partial acetone pressure, cm.	19.3	14.2	8.2	5.75
$T_{60}-T_{30}$, min.	1.2	2.6	9.1	42.5

	O_2/acetone = 3.1							
Partial acetone pressure, cm.	14.6	12.0	9.7	7.3	6.1	5.1	3.85	2.85
$T_{60}-T_{30}$, min.	1.7	2.5	4.0	7.5	13.5	17	38	71

	O_2/acetone = 3.65						
Partial acetone pressure, cm.	13.5	11.3	9.6	7.2	4.9	4.3	3.25
$T_{60}-T_{30}$, min.	1.7	2.5	3.8	7.0	13.5	22	39

	O_2/acetone = 4.35					
Partial acetone pressure, cm.	12.5	9.6	7.9	5.6	3.5	2.7
$T_{60}-T_{30}$, min.	1.4	2.75	4.6	9.8	20	36

	O_2/acetone = 5.00			
Partial acetone pressure, cm.	9.3	7.3	5.6	5.0
$T_{60}-T_{30}$, min.	2.7	6.6	9.7	14

TABLE VII

VALUES OF $T_{60}-T_{30}$ AT 450° C.

O_2/acetone = 2.00		O_2/acetone = 2.98		O_2/acetone = 4.60	
Partial acetone pressure, cm.	$T_{60}-T_{30}$, min.	Partial acetone pressure, cm.	$T_{60}-T_{30}$, min.	Partial acetone pressure, cm.	$T_{60}-T_{30}$, min.
18.4	0.36	16.1	0.24	10.7	0.74
12.8	0.95	14.2	0.47	8.2	0.95
9.3	2.05	9.5	1.20		
5.8	5.8	5.25	3.7		
		4.55	(2.5)		
		4.05	4.4		

TABLE VIII

VALUES OF $T_{50}-T_{30}$ AT 500° C.

	O_2/acetone = 2.16				
Partial acetone pressure, cm.	11.9	9.2	7.6	4.85	2.65
$T_{50}-T_{30}$, min.	0.19	0.20	0.25	0.64	1.52

Fig. 2 and the foregoing tables show that the rate of the reaction varies rapidly as the pressure varies for all temperatures and relative proportions of the reactants.

The order of the reaction will be given by the relationship

$$T_{50}-T_{30}=\frac{\text{constant}}{P^n-1},$$

where n is the order and P the initial pressure. For chain reactions the order has no very definite meaning, but it provides a convenient measure of the effect of pressure on the rate of the reaction. From the above relationship it may be seen that log $(T_{50}-T_{30})$ plotted against log P should give a straight line, from the slope of which the order may be calculated. A typical curve of this type is given in Fig. 6. From this curve the calculated order is 3.3. In Table IX a summary is given of the orders thus obtained for various mixtures and temperatures.

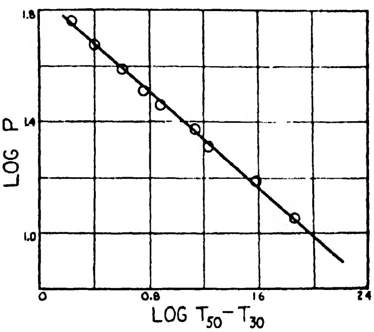

FIG. 6. *The logarithm of the total pressure plotted against the logarithm of the time for the reaction to proceed from 30 to 50%. 400°C.* 1 CH_3COCH_3+3.1 O_2.

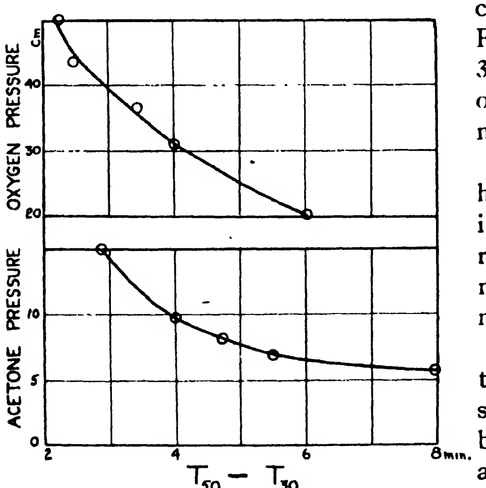

FIG. 7. *Influence of separate reactants on the rate of reaction. Upper curve: acetone, 10 cm.; oxygen varying. Lower curve: oxygen, 30 cm.; acetone varying.*

The order as shown in Table IX is high, not integral, and somewhat variable. This behavior is typical of a reaction which proceeds by a chain mechanism. Other evidence for such a mechanism will be discussed later.

The effect of a variation in the partial pressure of either constituent is shown in Fig. 7. It will be seen that both constituents affect the rate to approximately equal extents. Thus at 400° C. the rate may be approximately expressed by

$$-\frac{d}{dt}(CH_3COCH_3) = K\,(CH_3COCH_3)^{1.8}(O_2)^{1.8}.$$

TABLE IX

THE ORDER OF THE REACTION

Temp., °C.	350	350	350	400	400	400	400	400	450	450	500
O_2/acetone	2.00	3.03	4.95	2.03	3.10	3.65	4.35	5.00	2.00	2.98	2.16
Order	3.7	3.7	3.2	3.6	3.3	3.2	3.2	3.7	3.2	3.2	2.6

The Temperature Coefficient of the Reaction

In Fig. 8 $\log(T_{50}-T_{30})$ is plotted against the reciprocal of the absolute temperature for a reaction mixture with a partial oxygen pressure of 20 cm., and a partial acetone pressure of 10 cm. The heat of activation calculated from the slope of the line is 26,700 calories per gram molecule. The data from which Fig. 8 was constructed are given in Table X.

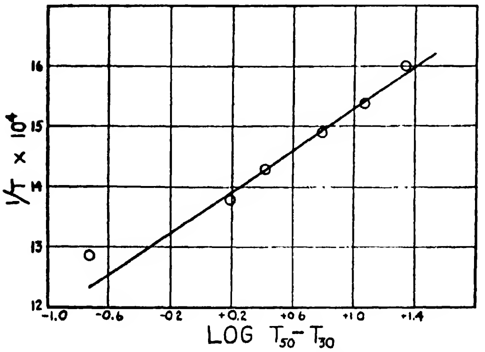

FIG. 8. *The temperature coefficient of the reaction.*

Since the order of the reaction does not vary much with temperature, the temperature coefficient cannot vary much with pressure. This was confirmed by plotting curves similar to the above.

TABLE X

THE TEMPERATURE COEFFICIENT OF THE REACTION

Temperature, °K.	632	648	673	698	723	773
$T_{50}-T_{30}$, min.	21.9	12.0	6.2	2.65	1.58	0.19

Partial oxygen pressure = 20 cm. Partial acetone pressure = 10 cm.

The linearity of the curve in Fig. 8 is only approximate. This is due to the varying relative importance of the induction period, which was mentioned previously (see Fig. 5). Very little weight can be given to the 350° C. point. The heat of activation can therefore be regarded only as an approximate indication of the effect of temperature on the reaction velocity.

The Effect of the Surface of the Reaction Vessel

The effect of surface was investigated by adding tubing to the reaction vessel. About three metres of Pyrex tubing, having an inner diameter of about 3 mm. was added to the vessel in 3-cm. lengths. This would have the effect of shortening the "free path" between the walls of the vessel to at most $\frac{1}{10}$ of its former value, and would increase the total surface about five times. A typical set of results for the packed tube are given in Table XI.

TABLE XI

TIMES FOR THE REACTION TO PROCEED FROM 30 TO 50% IN PACKED TUBE AT 400° C.

	O_2/acetone = 3.10			
Partial acetone pressure, cm.	15.5	10.2	9.2	5.0
$T_{50}-T_{30}$, min.	5.1	9.2	9.7	24.5

The pressure-time curves were entirely unaltered in form by the addition of the tubing, as shown in Fig. 9. There is, however, a decided retarding effect caused by the increase in surface. This effect is a well-known characteristic of a chain reaction. The magnitude of the retarding effect may be seen by comparing Table XI with Table VI. The retarding is much more pronounced at high pressures than at low. Thus at a partial acetone pressure of 5 cm., $T_{50}-T_{30}$ is about 1.4 times that for the unpacked tube: at 15.5 cm. it is about 3.2 times greater. This is to be expected, since in the absence of packing the wall factor would be much more pronounced at low pressures than at high. The change in retarding effect with pressure is well shown by calculating the order of the reaction in the packed tube. The order found is about 2.4, as compared with 3.3 for the unpacked tube. In other words, part of the effect of increased pressure on the rate is offset by the increased predominance of the wall factor.

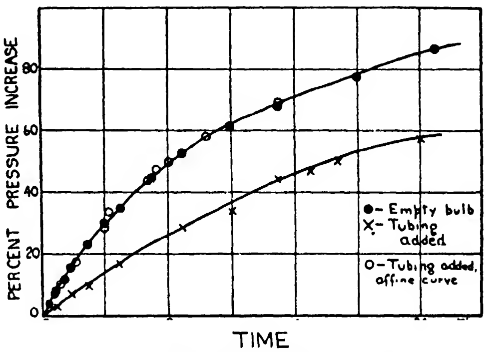

FIG. 9. *Affine curves with and without added tubing. 400° C. Partial acetone pressure, 10.2 cm.* $1CH_3COCH_3+3.0\ O_2$.

The magnitude of the retarding effect is small compared with that existing in many chain reactions. The indications, therefore, are that under normal conditions most of the chains are broken in the gas, the deactivating influence of the walls being comparatively small, and that the chains, therefore, are not of very great length.

The Effect of Added Nitrogen

Two experiments were carried out in the presence of added nitrogen. The results are shown in Table XII.

TABLE XII

THE EFFECT OF ADDED NITROGEN ON THE RATE OF THE REACTION

	$1\ CH_3COCH_3+3.10\ O_2+3.05\ N_2$. 400° C.	
Partial acetone pressure, cm.	12.0	7.2
$T_{50}-T_{30}$	1.5	8.3
$T_{50}-T_{30}$ for a similar mixture without nitrogen	1.7	8.0

Nitrogen, therefore, has no appreciable effect on the rate. This points to the same conclusion as in the previous section, *i.e.*, that the chains are comparatively short, and are broken mainly in the gas. If the wall factor were at all pronounced, nitrogen would be expected to increase the rate by keeping the chains off the walls. In the absence of any deactivation at the wall, nitrogen would be expected to have a small retarding effect due to its action as a diluent. The two effects are presumably about equally balanced.

Explosion Limits

A few experiments were made to determine if any concentrations existed at which there was a sharp transition from a slow change to an explosion. Such explosion limits have been shown by Hinshelwood and Thompson to occur with hydrogen-oxygen mixtures. No evidence was obtained for such limits in the oxidation of acetone. At high temperatures explosions occurred if the pressure were sufficiently high, but in all cases the reaction velocity increased continuously up to the point where explosion occurred. The explosions were therefore of the ordinary "thermal" type.

Discussion

In the foregoing sections it has been assumed that the oxidation of acetone proceeds by a chain mechanism. This assumption is supported by a number of facts: (i) the high and variable order, which is a characteristic of such reaction; (ii) the fact that an increase in the surface of the container causes a decrease in the rate; (iii) if the reaction had a real order as high as that found, *i.e.*, somewhat greater than three, it could not possess as high a heat of activation as 26,700 calories and yet proceed at a measurable rate.

The effect of nitrogen on the rate of reaction affords no evidence either for or against the existence of chains. If the reaction involves chains, a foreign gas would be expected to have a marked accelerating effect if the chains were normally broken at the wall. As previously pointed out, the absence of such an effect points to short chains which are mainly broken in the gas.

The temperature coefficient of the reaction throws some light on the mechanism of the process. The heat of activation found is 26,700 calories per gram molecule. A bimolecular reaction proceeding at the same rate would have a heat of activation in the neighborhood of 40,000 calories. In other words, the reaction proceeds more slowly than a bimolecular reaction with the same heat of activation. It would naturally be expected that a chain reaction would be faster than the corresponding bimolecular reaction.

It should be remembered, however, that there are indications that the reaction changes its course somewhat with changing temperature. In consequence, too much reliance must not be placed on any conclusions drawn from considerations of the heat of activation. The fact that the observed heat of activation is low may therefore be due to this cause, although it is doubtful if the change in the course of the reaction is sufficiently great to account for the discrepancy.

The low heat of activation might also be explained on the assumption that the process initiating the chains is termolecular rather than bimolecular. If

this were the case it would be necessary to postulate chains having a length of 10^3 to 10^4 molecules in order to explain the observed rate. This is out of the question in view of the evidence previously considered for short chains.

There is, however, no reason why the chain length should remain constant as the temperature is varied. If the chain length varies with temperature, the observed heat of activation will be a composite one, and will include the temperature coefficients of the initial process, and of the variation in chain length. Under these conditions no correlation will be possible between the heat of activation of the reaction, and that of a simple reaction.

The most probable explanation, therefore, would seem to be that the chains are short, that their length varies with temperature, and that the process initiating the chains is bimolecular.

The existence of an induction period must mean that products are accumulating in a manner which does not involve an increase in pressure. Egerton (2) suggests that the first stage in the oxidation of a hydrocarbon is the formation of an unstable peroxide, which provides the centre from which reaction chains commence. Fort and Hinshelwood (4) have given strong evidence that this is true of the oxidation of organic substances in general. Hatcher, Miss Howland, and the author (6, 7) have proved definitely that such a peroxide accumulates in the initial stages of the oxidation of gaseous acetaldehyde. It is therefore reasonable to assume that the induction period in the oxidation of acetone is due to the initial formation of a peroxide in the same way.

The oxidation of acetone differs from most oxidation reactions of the chain type in one important respect. The two reactants are of about equal importance in so far as the effect of their concentrations on the rate are concerned. They would therefore appear to be equally efficient in the propagation of chains.

References

1. Bone, W. A. and Townend, D. T. A. Flame and combustion in gases. London. 1927.
2. Egerton, A. C. and Gates, S. F. J. Inst. Petroleum Tech. 13: 281. 1927.
3. Fort, R. and Hinshelwood, C. N. Proc. Roy. Soc. London, A 127: 218-227. 1930.
4. Fort, R. and Hinshelwood, C. N. Proc. Roy. Soc. London, A 129: 284-290. 1930.
5. Gottlieb, L. Ann. 52: 121-130. 1844.
6. Hatcher, W. H., Steacie, E. W. R. and Howland, F. Can. J. Research, 5: 648-650. 1931.
7. Hatcher, W. H., Steacie, E. W. R. and Howland, F. Unpublished.
8. Hinshelwood, C. N. and Thompson, H. W. Proc. Roy. Soc. London, A 118: 170-183. 1928.
9. Hinshelwood, C. N. and Gibson, C. H. Proc. Roy. Soc. London, A 119: 591-606. 1928.
10. Hinshelwood, C. N. and Thompson, H. W. Proc. Roy. Soc. London, A 124: 219-227. 1929.
11. Holm, H. Z. angew. Chem. 26: 273-279. 1913.
12. Steacie, E. W. R. and Johnson, F. M. G. Proc. Roy. Soc. London. A 112: 542-558. 1926.
13. Thompson, H. W. and Hinshelwood, C. N. Proc. Roy. Soc. London. A 122: 610-621. 1929.
14. Thompson, H. W. and Hinshelwood, C. N. Proc. Roy. Soc. London. A 125: 277-291. 1929.
15. Wheeler, R. V. and Whitaker, A. J. Chem. Soc. 111: 267-272. 1917.
16. White, A. G. and Price, T. W. J. Chem. Soc. 115: 1462-1505. 1919.

STUDIES OF POLYMERS AND POLYMERIZATION

V. THE INFLUENCE OF METHYL AND PHENYL SUBSTITUTION ON THE POLYMERIZABILITY OF BUTADIENE[1]

BY GEORGE STAFFORD WHITBY[2] AND WILFRED GALLAY[3]

Abstract

A number of methyl- and phenyl- substituted butadienes were prepared and polymerized by different means under properly comparable conditions. The velocity and extent of polymerization were measured and the influence of the number and position of substituent groups on these two factors are discussed. The extent of polymerization depends upon the number of unsubstituted hydrogen atoms on the terminal carbon atoms of the conjugated system, and the ability of a conjugated diene to form a synthetic rubber depends upon the presence of at least three of these hydrogen atoms. The presence of phenyl substituents favors a tendency to dimer formation. The dimers from all the hydrocarbons were unsaturated ring compounds. 1-Phenyl-3-methyl butadiene and 1-3-diphenyl butadiene were found to polymerize spontaneously. The action of methyl magnesium iodide on benzalacetophenone results in both 1-2 addition to yield 1-3 diphenyl buten-1-ol-3, and in 1-4 addition to yield 2-4-diphenyl butanone-4, the former reaction preponderating.

In the last paper of the present series (26) a comparison of the polymerization of isoprene and 2-3-dimethylbutadiene-1-3 was recorded. In the present study a larger number of substituted butadienoid hydrocarbons, *viz.*, nine methyl-substituted and four phenyl-substituted hydrocarbons, has been examined, with the object of ascertaining the influence of the amount and kind of substitution on the ease and degree of polymerization.

The results make it clear that the presence of an unsubstituted terminal methylene group in these hydrocarbons is favorable to the occurrence of polymerization. They also indicate that in general increase in the number of substituent groups of a given kind reduces the ease of polymerization, especially to high molecular products.

Table I shows the results obtained in a comparison of the polymerization of all the five isomeric dimethylbutadienes under identical conditions.

The isomers fall in the same order as regards speed of polymerization in the experiments at both the temperatures employed. The isomer (2-3) in which there is no terminal substitution polymerizes most quickly and that (1-4) in which there is substitution at both ends of the butadienoid system least quickly. The *gem*-dimethyl compound (1-1) underwent polymerization to a greater extent but to a lower degree than the 1-4 compound, the "higher polymer" from it being only an oil of relatively low molecular weight. The "higher polymer" from the 2-3 and from the 1-3 compounds was an elastic, rubber-like solid. The 1-2 compound gave in the present experiments a firm gel which could perhaps hardly be described as rubber-like. A soft, rubbery polymer has however been obtained from this diene by Fisher (6).

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TABLE I

POLYMERIZATION OF THE DIMETHYLBUTADIENES-1-3 BY HEAT

	Hydrocarbon (Position of methyl groups)				
	2–3	1–2	1–1*	1–3	1–4
A. 30 Days at 100° C.					
Increase in d_4^{20}	—	0.0630	0.0669	0.0469	0 0197
Increase in d_D^{20}, %	—	8.5	9 5	6 5	2.8
Increase in n_D^{20}	0.0561	0.0185	0.0158	0 0084	0.0061
Increase in n_D^{20}, %	3.90	1 21	1.09	0.59	0 42
Total polymerization, %	100	91	57	51	33
Dimer formed, %	54	53	43	32	20
Higher polymers, %	46	38	14	19	32
Mol. wt. of higher polymer	1377	919	432	1334	795
B. 15 Days at 150° C.					
Increase in d_4^{20}	0.1360	0 1172	0 0972	0.0968	0.0789
Increase in d_D^{20}, %	17.2	15.7	13.6	13.3	10.9
Increase in n_D^{20}	0.0439	0.0327	0.0267	0 0215	0.0171
Increase in n_D^{20}, %	3.05	2.25	1.85	1.49	1.17
Total polymerization, %	100	100	83	78	68
Dimer formed, %	63	73	59	59	51
Higher polymers, %	37	27	24	19	17
Mol. wt. of higher polymers	1184	674	304	1002	602

**See remarks in the experimental part concerning the chemical identity of this preparation.*

It may be noted that Lebedev (15) found the terminally substituted piperylene (1-methylbutadiene) to polymerize less readily than isoprene (2-methylbutadiene). On heating at 150° C. for 15 hr. he found these hydrocarbons to undergo polymerization to the extents of 30% and 79% respectively.

The polymerization of all five dimethylbutadienes at both 100° C. and 150°C. leads to the formation of a large amount of oily dimer. The proportion of dimer to higher polymer formed is greater at the higher than at the lower temperature (cf. 26). The 1-1 and 1-2-dimethylbutadienes yielded two dimers. In the case of each of the other three dimethylbutadienes the evidence indicated that only one dimer was formed. Determinations of the degree of unsaturation indicated that all the dimers were ring compounds and hence incapable of further polymerization. The dimers of 2-3-dimethylbutadiene have previously been examined by Whitby and Crozier (26) and those of isoprene have been

examined with great care by Whitby and Crozier (26) and by Wagner-Jauregg (24). They were found to consist of ring compounds, open-chain dimers being absent.

Experiments on the polymerization of two trimethylbutadienes, namely, the 1-1-3 and 1-1-4 compounds showed (a) that terminal substitution retards polymerization, since the latter compound showed a smaller tendency to polymerize than the former, (b) that the additional substitution, as compared with that in the dimethylbutadienes, retards polymerization. While sulphuric acid polymerized 1-1-3-trimethylbutadiene to the extent of 81%, yielding polymeric material as high in part as a pentamer, it was without action on 1-1-4-trimethylbutadiene under the same conditions. When polymerized by means of heat, the two isomers gave the results shown in Table II.

TABLE II
POLYMERIZATION OF TRIMETHYLBUTADIENES

Heat treatment	Total polymerization, %	Dimer, %	Higher polymer, %	Mol. wt. higher polymer
A. 1-1-3-Trimethylbutadiene				
13.5 days at 85° C.	10	1	9	—
28 days at 85° C.	14	1	13	348
30 days at 85° C. and 38 days at 140° C.	63	35	28	374
B. 1-1-4-Trimethylbutadiene				
13.5 days at 85° C.	Trace	0	Trace	
38 days at 140° C.	24	20	4	

It will be noted that the 1-1-3 compound polymerizes less than either the 1-1 or 1-3 dimethyl compounds, and similarly that the 1-1-4 compound polymerizes less than the 1-1 or 1-4 dimethyl compounds.

Experiments with two tetramethylbutadienes also show that terminal substitution retards polymerization, and, when the results are compared with those for the di- and tri-methyl compounds, that increase in the degree of substitution is unfavorable to polymerization. The 1-1-4-4 compound, in which all the terminal hydrogens of the butadiene chain are substituted, was considered by Pribytek (19) to show inclination to polymerize and has been mentioned in the patent literature as polymerizable (4), but Lebedev (15) found it to yield only a trace of polymer when heated for 90 days at 150° C. On the other hand, 1-2-3-4-tetramethylbutadiene, which was first prepared by Macallum and Whitby (17) and found to undergo very little change in four days at 100° C., has, in the present experiments, been found to polymerize to the extent of 80% in five days at 235° C., nearly half the polymeric product having a molecular weight corresponding to a pentamer.

Just as 1-phenyl butadiene polymerizes much faster than butadiene, so

1-phenyl-3-methyl butadiene (phenyl isoprene) has been found to polymerize much more quickly than isoprene. In fact no unpolymerized hydrocarbon could be isolated from the preparation. Grignard (7, p. 486) and Klages (11) also have noted that this hydrocarbon polymerizes quickly on heating. The dimer produced possesses a ring structure and was found incapable of undergoing further polymerization. Results given in the literature indicate that although phenyl-substituted butadienes polymerize more readily than corresponding methyl-substituted compounds, they polymerize generally to ring dimers only; in no case have they yielded high, rubber-like polymers.

2-3-Diphenyl butadiene has been reported to polymerize rapidly at room temperature (10, 22). 1-3-Diphenyl butadiene (hitherto unknown) showed such remarkable tendency to undergo polymerization that the latter reaction took place partially in cold ethereal solution, the insoluble dimer being precipitated. No unpolymerized hydrocarbon was recovered and no polymer other than the dimer was isolated. Attempts to polymerize this dimer further by various means were unsuccessful. The action of methyl magnesium iodide on benzalacetophenone resulted in both 1-2 addition to yield the corresponding tertiary alcohol and in 1-4 addition to yield the corresponding saturated ketone.

$$CH_3MgI + C_6H_5CH{=}CHCOC_6H_5 \longrightarrow (1)\ C_6H_5CH{=}CH\underset{\substack{|\\ C_6H_5}}{C}{=}CH_2 \qquad 70\%\ \text{yield}$$

$$(2)\ C_6H_5\,\underset{\substack{|\\ CH_3}}{C}HCH_2COC_6H_5 \qquad 21\%\ \text{yield}$$

In contrast to the 2-3 and 1-3 compounds, 1-4 diphenyl butadiene, with its two terminal substituents, was found to be stable at temperatures below the melting point (150° C.) and only above that temperature did polymerization take place. By heating for five days at 235° C., there was obtained a gelatinous polymer of low degree of polymerization and by treatment with antimony pentachloride was obtained an amorphous gray powder, mol. wt. 962.

The isomeric 1-2-3-4 and 1-1-4-4 tetraphenyl butadienes were found to be but little affected by heating for five days at 235° C. By the action of antimony pentachloride, the 1-1-4-4 compound, in which all the terminal hydrogens of butadiene are substituted, remained practically unchanged whereas the 1-2-3-4 compound yielded a polymer of mol. wt. 1090.

Although the present study does not afford any conclusive evidence as to the mechanism of the polymerization of methyl-substituted butadienes to high polymers and as to the structure of the latter, it is not without a bearing on this question.

Staudinger considers the polymerization of isoprene to caoutchouc to involve the mutual, partial 1-4 addition of isoprene molecules to form long chains with free terminal valencies (21). The following scheme shows the application of Staudinger's view to 2-3-dimethylbutadiene.

$$\ldots.CH_2.CMe{:}CMe.CH_2\ldots + [\ldots..CH_2.CMe{:}CMe.CH_2\ldots]_n + \ldots CH_2.CMe{:}CMe.CH_2\ldots.$$

$$\downarrow$$

$$\ldots CH_2.CMe{:}CMe.CH_2.[CH_2.CMe{:}CMe.CH_2]_n.CH_2.CMe{:}CMe.CH_2\ldots.$$

According to this scheme of polymerization, no wandering of hydrogen atoms takes place. On the other hand, the scheme of polymerization of isoprene to caoutchouc which has been advanced by Whitby (25), as an outcome of earlier studies in this series (27, 28), involves as an essential feature the wandering of hydrogen. According to this scheme the polymerization of conjugated dienes to high polymers involves the addition of successive molecules with wandering of a terminal hydrogen atom at each step. In view of the fact that the present study shows the importance of free terminal hydrogen atoms in the polymerization of substituted butadienes in favoring polymerization, it may be said that the results of this study accord well with such a scheme of polymerization. Complete terminal substitution, as in 1-1-4-4-tetramethyl and -tetraphenyl butadienes, prevents polymerization altogether; partial substitution of the terminal hydrogens retards it, possibly through steric hindrance.

Applied to 2-3-dimethylbutadiene, the scheme of Whitby may be shown as follows. Since the main product of ozonolysis is acetonyl-acetone (8, 9) it is clear that the addition takes place in the 1-4, not the 1-2, sense.

$$CH_2{:}CMe.CMe{:}CH_2 + \boxed{H}.CH{:}CMe.CMe{:}CH_2 \longrightarrow CH_3.CMe{:}CMe.CH_2.CH{:}CMe.CMe{:}CH_2$$

$$\downarrow +C_6H_{10}$$

$$CH_3.CMe{:}CMe.CH_2.CH_2.CMe{:}CMe.CH_2.CH{:}CMe.CMe{:}CH_2$$

$$\downarrow +(n-1)C_6H_{10}$$

$$CH_3.CMe{:}CMe.CH_2.[CH_2.CMe{:}CMe.CH_2]_n.CH{:}CMe.CMe{:}CH_2$$

Experimental

Dimethylbutadienes

2-3-Dimethylbutadiene. This was prepared by the dehydration of pinacone by means of hydrobromic acid. B.p., 69-70° C.; n_D^{20}, 1.4375; d_4^{20}, 0.7263; yield, 80%.

1-3-Dimethylbutadiene. The tertiary alcohol, 4-methyl penten-2-ol-4, prepared from ethylidene acetone and methyl magnesium iodide, was dehydrated by means of hydrobromic acid (13). B.p., 76-77° C.; n_D^{20}, 1.4418; d_4^{20}, 0.7215.

1-2-Dimethylbutadiene. Tiglic aldehyde (16) was prepared by heating freshly prepared acetaldehyde and propionaldehyde in aqueous sodium acetate solution for 28 hr. at 100° C. in an autoclave fitted with a stirrer. After rising to 95 lb. per sq. in., the pressure slowly dropped to below atmospheric. The aldehyde layer was separated and the by-products, consisting mainly of crotonaldehyde and methylethylacrolein, removed by fractionation in an atmosphere of carbon dioxide. Yield, 40% based on the propionaldehyde; b.p., 114-117° C. By treatment of the tiglic aldehyde with methyl magnesium iodide, the secondary alcohol, 3-methyl penten-2-ol-4, was obtained (1). B.p., 88-90° C./115 mm.; yield, 75%. The alcohol was dehydrated to the desired hydrocarbon by means of hydrobromic acid. Dehydration of the secondary alcohol occurred with greater difficulty than in the succeeding case. B.p., 76-79° C.; n_D^{20}, 1.4528; d_4^{20}, 0.7452.

1-4-Dimethylbutadiene. The secondary alcohol, hexen-2-ol-4, was prepared from carefully purified crotonaldehyde and methyl magnesium iodide (20). B.p., 85-87° C./118 mm.; yield, 85%. Dehydration by hydrobromic acid gave the desired hydrocarbon in 65% yield. (Potassium bisulphate gave only a 10% yield.) B.p., 80-82° C.; n_D^{20}, 1.4502; d_4^{20}, 0.7167.

1-1-Dimethylbutadiene. Diacetone alcohol was reduced to 2-methyl pentandiol-2-4 by means of sodium amalgam (29). The crude diol (65% yield) was purified by fractionation at 1 mm. (50% yield). It was dehydrated by means of hydrobromic acid (14), after potassium acid sulphate had been found to give very poor yields. The diol (100 cc.) was heated for three hours under a Hempel column with 2 cc. 48% HBr and pumice, the heating being controlled so as to keep the vapor temperature below 95° C. A further 100 cc. of the diol was then added and heating continued, a little more HBr being added during the latter part of the operation to replace loss. The resultant clear liquid was separated from the aqueous layer, dried over potassium carbonate and fractionally distilled. The product was dried over calcium chloride and again fractionated. B.p., 76-77° C.; n_D^{20}, 1.4472; d_4^{20}, 0.7204; yield in the dehydration, 30%.

Since this work was done an examination of the hydrocarbon obtained by the dehydration of the glycol in question has been carried out by Diels and Alder (3, pp. 68-73, 98-101) and by Farmer, Lawrence and Scott (5), who conclude that the product is 1-3-, not 1-1-, dimethylbutadiene. The product obtained by the present authors, however, differs from the 1-3 isomer noticeably in density and refractive index (*vide supra*) and, above all, in its behavior on polymerization. In polymerization experiments with the authors' preparations there is a noticeable difference in the speed of polymerization (see Table I) and in the nature of the dimeric oily products, and there is a very great difference in the character of the higher polymer formed, that from the 1-3 isomer being rubber-like, while that from the 1-1 isomer was an oil. It must be considered probable that while, as Diels and Alder's investigations indicate, the hydrocarbon product obtained by the dehydration of the glycol prepared by the reduction of acetone alcohol, contains some 1-3-dimethylbutadiene, yet, as employed in the present study, it was preponderantly the 1-1 compound.

Polymerization by heat. Each hydrocarbon (10 gm.), freshly distilled, was heated in sealed tubes of approximately the same capacity, for 15 days at 150° C. and for 30 days at 100° C. The density and refractive index after heating were measured in each case. The contents of each tube was distilled, unchanged monomer being first removed at atmospheric pressure, and dimeric products then separated under reduced pressure. In no case could any product higher than a dimer be distilled off without decomposition. The higher polymeric material remaining after the distillation was purified by precipitation with alcohol from solution in benzene. After two repetitions of this purification process and drying *in vacuo*, the molecular weight of the higher polymer was determined cryoscopically in benzene at the same concentration in all cases.

The main results of these comparative polymerization experiments have been given in Table I. The results of an examination of the dimeric products are recorded below.

Inspection of the tubes during the periods of heating indicated the differences in the rates of polymerization of the isomers which the data in Table I reveal in quantitative terms. At 150° C., after three days the 2-3 compound had changed to a very viscous oil; after seven days this compound had become a barely fluid, glass-like mass, while the 1-2 compound appeared more viscous than originally and the other compounds had not noticeably increased in viscosity. At 100° C., after 15 days the compounds were similar in appearance to what they were after seven days at 150° C.

The higher polymeric material isolated at the end of the heating periods had the following character. From the 2-3 and 1-3 compounds it was a white, elastic solid, more readily soluble in benzene in the latter than in the former case. From 1-2-dimethylbutadiene the higher polymer obtained at 100° C. was a rather oily, firm gel, while that obtained at 150° C. was a gelatinous sticky solid. It is not improbable that at a lower temperature than 100° C., the 1-2 isomer would yield a rubber-like product. Indeed Fisher (6) has reported that he obtained a soft rubbery product from this diene by heating at 100° C. with benzoyl peroxide or in the presence of air. From the 1-4 compound the product at 100° C. was a softer gel than from the 1-2 compound; and at 150° C., a viscous oil. The 1:1 compound gave only an oil. The latter was not purified for the molecular weight determination, as it could not be reprecipitated from solution.

Dimers. Dimers isolated after the heat polymerization of the dimethylbutadienes were as follows.—

(1) 2-3-Dimethylbutadiene gave only one dimer. B.p., 95° C./17 mm.; n_D^{20}, 1.4815; mol. wt., 167 (calc., 164). Iodine absorption per mol., 1.82 mol.

(2) 1-2-Dimethylbutadiene gave two dimers, the second in very small amount only.

(a) B.p., 97-100° C./16 mm.; n_D^{20}, 1.4815; mol. wt., 165 (calc., 164). Iodine absorption: 1.73 mol. per mol.

(b) B.p., 108-110° C./16 mm.; n_D^{20}, 1.4850.

(3) 1-1-Dimethylbutadiene gave two dimers, the higher boiling one in very small amount:

(a) B.p., 94-97° C./16 mm.; n_D^{20}, 1.4752; mol. wt., 164. Iodine absorption: 1.71 mol. per mol.

(b) B.p. 105-108° C./16 mm.; n_D^{20}, 1.4800.

(4) 1-3-Dimethylbutadiene gave only one dimer. B.p. 90-92°C./16 mm.; n_D^{20}, 1.4758; mol. wt., 167. Iodine absorption: 1.94 mol. per mol.

(5) 1-4-Dimethylbutadiene gave two dimers, the higher boiling one in very small amount.

(a) B.p. 90° C./20 mm.; n_D^{20}, 1.4695; mol. wt., 166. Iodine absorption: 1.90 mol. per mol.

(b) B.p. 96-98° C./20 mm.

The iodine absorption was determined by means of Hanus solution applied in 50% excess for 24 hr. The results, although consistent, were somewhat low, possibly due to the time allowed for absorption being insufficient. Concordant results could not be obtained with a solution of bromine in chloroform, although this reagent was used successfully with other dimers (*infra*).

Polymerization by catalysts. To 5 cc. of each of the dimethylbutadienes was added 3 cc. of a 20% solution of stannic chloride in chloroform. Much heat was evolved and highly colored solutions were obtained. After four hours' standing, the solutions were diluted with 10 cc. of chloroform and were poured into a large excess of absolute alcohol. Only from 2-3-dimethylbutadiene was a solid polymeric product obtained; from the other isomers the precipitate consisted of a small amount of a reddish oil containing halogen and of about the same specific gravity as the chloroform-alcohol mixture.

1-1-3 and 1-1-4 Trimethyl butadienes. 2-4-Dimethyl penten-2-ol-4, prepared by the action of methyl magnesium iodide on mesityl oxide, was dehydrated to 1-1-3-trimethyl butadiene-1-3 by distillation under atmospheric pressure*. B.p., 92° C./749 mm. Grignard (7, p. 478) gives the boiling point as 92-93° C./750 mm. Yield, 50% based on mesityl oxide used.

5-Methyl hexen-2-ol-4 was prepared by the action of isopropyl magnesium bromide on crotonaldehyde (20). The yield of redistilled alcohol (B.p. 90-93° C./100 mm.) was 60% based on the crotonaldehyde used. By the use of 48% hydrobromic acid dehydration to 1-1-4-trimethyl butadiene-1-3 was effected in 55% yield as compared with 10% yield using $KHSO_4$. B.p., 97-99° C.; n_D^{20}, 1.4585; d_4^{20}, 0.7461.

Polymerization of the trimethyl butadienes by sulphuric acid. The same procedure was used for each of the hydrocarbons. The hydrocarbon (10 gm.) was added dropwise with stirring to 90 gm. of 80% sulphuric acid cooled in ice. After standing at 0° C. for ½ hr., with frequent shaking, the reaction mixture was poured on chipped ice and the whole extracted three times with ether. The ethereal solution was washed with sodium bicarbonate and then with water. It was dried over anhydrous sodium sulphate and the ether removed.

In the case of the 1-1-4 isomer, the remaining liquid was distilled under atmospheric pressure and the original hydrocarbon obtained in almost 100% recovery.

In the case of the 1-1-3 isomer, 9.1 gm. of a viscous, colorless oil remained which was fractionally distilled. After removal of the monomer, distillation under reduced pressure yielded two fractions besides a small amount of a non-distillable residue. Total polymerization, 81%: 1st fraction, 60; 2nd fraction, 11; Residue, 10.

Fraction I—B.p. 87-89° C./8 mm., n_D^{20}, 1.4804, mol. wt. 185 (calc. for dimer, 192). Unsaturation determination (Hanus method) showed 1.86 mol. iodine absorbed per mol. of hydrocarbon. A dimer prepared by the action of 80% sulphuric acid on the diene is described by Grignard (7, p. 478): b.p., 98-100°

**For this preparation the authors are indebted to R. N. Crozier.*

C./12 mm.; n_D^{10}, 1.48483. Fraction II—B.p. 170-175° C./4 mm., mol. wt. 282 (calc. for trimer 288); C, 86.8; H, 12.2% (calc. for polymer: C, 87.5; H, 12.5%). Mols. iodine absorbed per mol. of hydrocarbon, 1.80 (Hanus method). Residue—Mol. wt. 471 (calc. for pentamer 480).

Polymerization of the trimethyl butadienes by heat. (a) Three samples of 1-1-3-trimethylbutadiene were heated under conditions listed and then remained sealed at room temperature for about one year before examination. Fractional distillation yielded the following results which have been recorded in Table II. The dimer has been described above. The higher polymer from Sample No. 1 was a viscous, gelatinous oil, mol. wt. 374 (calc. for tetramer, 384); C, 87.8; H, 12.2% (calc. 87.5, 12.5%). Mol. wt. of the higher polymer from Sample No. 2 was 348.

(b) Two samples of 1-1-4 trimethylbutadiene were heated under conditions listed and then fractionally distilled. Results have been given in Table II. The dimer was a colorless liquid; B.p., 92-95° C./8 mm.; n_D^{20}, 1.5037; mol. wt., 198 (calc. 192). Absorption of iodine: 1.90 mol. per mol.

(c) By the action of stannic chloride and antimony pentachloride on 1-1-4 trimethylbutadiene as described in the case of the dimethyl butadienes, there were obtained only small amounts of reddish oils containing halogen and of about the same specific gravity as the chloroform-alcohol mixture.

1-2-3-4 Tetramethylbutadiene-1-3. Methyl ethyl ketone was reduced to 3-4 dimethyl hexandiol-3-4 by amalgamated magnesium in benzene solution (17). B.p. 124-127° C./55 mm.; yield, 30%. By means of a trace of dilute sulphuric acid the diol was dehydrated to the desired hydrocarbon. B.P., 71-73° C./100 mm.; yield, 75%.

A sample of the hydrocarbon was heated in a sealed tube for five days at 235° C. and then fractionally distilled. The total polymerization was 80%, of which 45% was Fraction 1, 20% Fraction 2 and 15% undistillable residue. Fraction 1—B.p., 127-123° C./14 mm.; n_D^{20}, 1.4900; mol. wt., 229 (calc. for dimer 220); iodine absorption, 2.14 mol. per mol. of hydrocarbon. Fraction 2—B.p., 170-180° C./10 mm.; mol. wt., 304 (calc. for trimer 330); iodine absorption, 1.74 mol. per mol. Residue—Mol. wt., 504 (calc. for pentamer 550). On treatment with stannic chloride and antimony pentachloride the diene gave only small amounts of colored oils containing halogen.

1-3-Diphenyl butadiene-1-3. The method attempted for the preparation of the hydrocarbon was the interaction of methyl magnesium iodide and benzylidene acetophenone to yield the corresponding tertiary alcohol with subsequent dehydration of the latter. The product proved to be a dimer of the desired hydrocarbon.

Benzalacetophenone was prepared by the condensation of benzaldehyde and acetophenone in alkaline solution. The ethereal solution of the benzalacetophenone was added very slowly to the methyl magnesium iodide, with strong stirring to avoid local heating, and the temperature of the reacting mixture was kept at −10° C. After hydrolysis of the complex, the ethereal solution was washed successively with 5% sodium hydroxide, sodium bisulphite and

water, dried over anhydrous sodium sulphate, and the ether was removed. The resultant liquid was allowed to stand overnight when a considerable mass of light yellow solid containing a small amount of an oil separated out. The precipitate was collected and washed repeatedly with alcohol warmed to about 40° C. The solid was recrystallized from alcohol (the treatment of the filtrate is described in a later section). Yield: 70%. The recrystallized material consisted of very fine, white crystals, m.p. 167° C., soluble in benzene, chloroform and boiling alcohol. C, 93.8; H, 6.9% (calc. for $C_{16}H_{14}$: C, 93.2; H, 6.8%); mol. wt., 414 (calc. for a dimer of this hydrocarbon; 412). Two unsaturation determinations, carried out with a bromine solution in chloroform, gave 2.09 and 2.05 mol. bromine absorbed per mol. of hydrocarbon.

Small amounts of this ring dimer were sealed in tubes and heated for varying lengths of time, but in all cases the dimer was recovered practically unchanged. Stannic chloride and antimony pentachloride had no action on a chloroform solution of the dimer beyond developing a slight greenish color.

It was expected that the filtrate after separation of the dimer would contain essentially the desired alcohol, 1-3-diphenyl buten-1-ol-3. Accordingly the oil was refluxed with an excess of acetic anhydride for 2 hr. No precipitate appeared on cooling. The oil was taken up in ether, washed with dilute sodium hydroxide, then with water, and dried over anhydrous sodium sulphate. The ether was removed, and the oil allowed to stand for several days in the cold, when crystallization took place. Yield: 21%. The crystals were soluble in all organic solvents. They were finally recrystallized from 50% methyl alcohol with much loss. The product appeared to be 1-3-diphenyl butanone-1. M.p., 74° C.; mol. wt., 224 (calc. for $C_{16}H_{16}O$, 224); C, 85.8; H, 7.3% (calc. for this ketone: C, 85.7; H, 7.1%).

1-Phenyl 3-Methyl butadiene 1-3. Recrystallized benzal acetone in ethereal solution was added dropwise with stirring to a solution of methyl magnesium iodide at −10° C. After hydrolysis of the Grignard complex, the ethereal extract was separated, washed successively with dilute sodium hydroxide solution, sodium bisulphite solution and finally with water. After drying over anhydrous potassium carbonate, the ether was removed, a yellow oil remaining. Without further purification this oil was dehydrated. It was treated with an excess of acetic anhydride and the solution heated over a boiling water bath for three hours. Then the solution was distilled under 150 mm. pressure and the acetic anhydride and acetic acid removed. According to Grignard (7, p. 486), the desired hydrocarbon boils at 115° C./18 mm. (*cf.* 11). However, in this instance, no distillate was obtained, even after lowering the pressure to 4 mm. and raising the temperature to 160° C. It was concluded, then, that no monomeric hydrocarbon was present and that the resulting viscous brown liquid contained the hydrocarbon in polymerized form. The material was very soluble in benzene and chloroform, and alcohol failed to throw out any product, indicating the absence of any high polymer.

The oil (35 gm.) was then distilled from a Claisen flask in an atmosphere of carbon dioxide. A few drops distilling at 100° C./2 mm. were discarded. A

clear light yellow oil (10 gm.), distilling at 180-185° C./2 mm., was collected. No further distillate could be obtained without decomposition. Mol. wt. 296 (calc. for dimer of 1-phenyl 3-methyl butadiene, 288): C, 92.02; H, 8.00% (calc. for above dimer, C, 91.7; H, 8.3%). In two determinations 1.88 and 1.93 mol. respectively of bromine was absorbed per mol. of hydrocarbon from a solution of bromine in chloroform.

It was attempted to polymerize further this ring dimer by heating small amounts in sealed tubes for 15 days at a temperature of 250° C., with and without sodium. In all cases the dimer remained unchanged. Heating at 100° C. with benzoyl peroxide produced no change. Stannic chloride did not exert any apparent effect, no heat being evolved and no color change apparent.

1-4-Diphenylbutadiene-1-3 (*trans-trans*). The hydrocarbon was prepared by the condensation of cinnamic aldehyde and phenyl acetic acid according to the procedure of Kuhn and Winterstein (12, p. 103). After recrystallization from acetic acid, there were obtained light yellow large crystals showing green and blue fluorescence, m.p. 150° C.

The hydrocarbon (10 gm.) dissolved in 150 cc. chloroform was treated with 10 cc. of a 20% solution of anhydrous antimony pentachloride in chloroform. The solution became warm and turned violet in color. After three days' standing it was poured into a large excess of absolute alcohol. The precipitate, about 2 gm. of a gray amorphous powder, was purified by reprecipitation twice by absolute alcohol from benzene solution. M.p. 243-250° C.; mol. wt., 962 (calc. for pentamer, 1020). A sample of the hydrocarbon was heated for five days, at 235° C. There was produced a gelatinous viscous oil, readily soluble in benzene.

1-2-3-4 Tetraphenyl butadiene-1-3 and 1-1-4-4-tetraphenyl butadiene-1-3. Desoxy benzoin pinacone, prepared by the reduction of benzoin by zinc dust in acetic acid (2), was dehydrated to the first-mentioned hydrocarbon by means of acetyl chloride (18). The product was recrystallized from glacial acetic acid. M.p., 183° C. Yield on dehydration, 30%.

In order to obtain the second-mentioned hydrocarbon, tetraphenyl tetramethylene glycol was prepared by the action of phenyl magnesium bromide on ethyl succinate in ethereal solution (23). After hydrolysis of the complex and separation of the ether layer, the required glycol precipitated from the latter in the cold on standing. M.p., 206° C.; yield, 70%. Dehydration was effected by boiling in glacial acetic acid solution with a large excess of conc. HCl. M.p., 202° C.; yield on dehydration, 90%.

Polymerization by antimony pentachloride. Each hydrocarbon (10 gm.) was dissolved in 120 cc. of chloroform and 10 cc. of a 20% anhydrous halide solution in chloroform added. In the case of the 1-2-3-4 compound, the reaction mixture became warm and turned deep violet in color. The 1-1-4-4 isomer developed little heat of reaction with only a faint blue color. After standing for three days, the solutions were poured into a large excess of absolute alcohol and the resultant precipitates purified by twice dissolving in chloroform and precipitating with alcohol. The products were then twice extracted with

boiling glacial acetic acid in order to remove unchanged monomer. The product from the 1-1-4-4 isomer was totally soluble in this medium and when recrystallized from it melted at 197° C. (m.p. monomer, 202° C.), mol. wt. 372 (calc. for monomer, 358). In the case of the 1-2-3-4 isomer, a small amount of a reddish-yellow amorphous powder remained after the acetic acid extraction. Heating to 350° C. left the solid unmelted. Mol. wt. 1090 (calc. for trimer 1074).

Polymerization by heat. A sample of each hydrocarbon was heated in a sealed tube at 235° C. for five days. The products were dissolved in benzene and precipitated with alcohol. The product from the 1-2-3-4 diene was a sticky, glass-clear, viscous oil, and that from the 1-1-4-4 diene, a reddish-yellow powder, formed in very small amount. The molecular weights were but slightly changed from those of the monomers, being 559 and 590 for the 1-2-3-4 and 1-1-4-4 products respectively.

References

1. Abelmann, P. Ber. 43: 1574-1588. 1910.
2. Blank, A. Ann. 248: 1-34. 1888.
3. Diels, O. and Alder, K. Ann. 470: 62-103. 1929.
4. Farbenfabriken vorm. Friedr. Bayer & Co. D.R.-P. 235, 686.
5. Farmer, E. H., Lawrence, C. D. and Scott, W. D. J. Chem. Soc. 510-530. 1930.
6. Fisher, H. L. Ind. Eng. Chem. 22: 869-871. 1930.
7. Grignard, V. Ann. Chim. Phys. 24 (7): 433-490. 1901.
8. Harries, C. Ann. 383: 157-227. 1911.
9. Harries, C. Ann. 395: 211-272. 1913.
10. Johlin, J. M. J. Am. Chem. Soc. 39: 291-293. 1917.
11. Klages, A. Ber. 35: 2649-2652. 1902.
12. Kuhn, R. and Winterstein, A. Helv. Chim. Acta. 11: 87-116. 1928.
13. Kyriakides, L. P. J. Am. Chem. Soc. 36: 657-663. 1914.
14. Kyriakides, L. P. J. Am. Chem. Soc. 36: 987-1005. 1914.
15. Lebedev, S. V. and Merezhkovskii, B. K. J. Russ. Phys. Chem. Soc. 45: 1249-1388. 1914.
16. Leiben, A. and Zeisel, S. Monatsh. 7: 53-74. 1886.
17. Macallum, A. D. and Whitby, G. S. Trans. Roy. Soc. Can. 22: III; 39-44. 1928.
18. Orechoff, A. Ber. 47: 89-95. 1914.
19. Pribytek, S. J. Russ. Chem. Soc. 20: 506-512, 1888. J. Chem. Soc. Absts. 56: 362. 1889.
20. Reif, J. Ber. 41: 2739-2746. 1908.
21. Staudinger, H. and Fritschi. J. Helv. Chim. Acta. 5: 785-806. 1922.
22. Thörner, W. and Zincke, T. Ber. 13: 641-647. 1880.
23. Valeur, A. Bull. soc. chim. 29: 683-689. 1903.
24. Wagner-Jauregg. T. Ann. 488: 176-185. 1931.
25. Whitby, G. S. Trans. Inst. Rubber Ind. 5: 184-195. 1929.
26. Whitby, G. S. and Crozier, R. N. Can. J. Research 6: 203-225. 1932.
27. Whitby, G. S. and Katz, M. J. Am. Chem. Soc. 50: 1160-1171. 1928.
28. Whitby, G. S. and Katz, M. Can. J. Research, 4: 344-360. 1931.
29. Zelinsky, N. and Zelikow, J. Ber. 34: 2856-2867. 1901.

A QUANTITATIVE METHOD FOR MEASURING THE DETERGENT ACTION OF LAUNDRY SUPPLIES[1]

By O. M. Morgan[2]

Abstract

An improved type of standard soil for measuring and comparing the detergent efficiencies of soaps has been developed. The technique of application of this soil to white, desized cotton sheeting has been studied with respect to obtaining a uniform product which is sensitive to small differences in washing procedure, both chemical and mechanical. The effects of time, temperature, soap concentration, and various rates of agitation have been investigated in connection with this soil. The results prove its sensitivity.

A miniature wash wheel has been constructed for use in the above work. It exhibits several improvements over small machines of this type formerly used.

Soap is recognized as the primary cleansing agent and the fundamental laundry supply. Statistics for the Dominion of Canada for the year 1929 show that approximately three millions of dollars was spent in the purchase of this product for commercial laundry purposes alone. This figure represents an increase over the previous year of 13.2%. A rapidly growing industry such as laundering requires scientific guidance in the proper channels. One of its most important problems is the choice of proper and efficient supplies. When these are obtained the next step is their efficient use to produce quality washing.

The science of laundering is one which contains many variables. Climatic conditions and geographic situation cause the type of soil on the fabrics to vary. Varying degrees of hardness of the water used offered difficulties to the industry until the introduction of lime-soda and zeolite water softeners. The art of stain removal has been studied very carefully and is now on a fairly satisfactory basis. However, no method has been evolved which has attained general acceptance for the quantitative comparison of one soap with another with respect to their washing powers. In order to determine and compare the washing powers of several soaps, a washing operation must now be actually carried out. The literature contains several papers recording experiments in which various physical properties of soap solutions were measured and the results interpreted to indicate detergent efficiency. The physical properties which have been measured in this connection are surface tension against air or oils, lathering power, emulsifying power, power of soap additions in the stabilizing of other emulsions or suspensions, and more recently the effect of the pH values. It has been definitely shown that these circuitous methods of arriving at detergent power, while being a certain amount of assistance, are not entirely satisfactory.

Previous Work

The literature on this subject up to 1928 is well reviewed in a paper by Rhodes and Brainard (6). The Detergents Committee of the American Oil Chemists Society has been working for several years in an effort to develop a

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test which will evaluate and compare detergent materials used in laundry practice. Their progress to date is reported by Vail (8). At present umber is being used as a soiling agent and is applied either in a Launderometer, or by dipping the samples, passing them through a wringer, and then rinsing in successive portions of clear water until the liquid remains clear. Washing is effected in a Launderometer and color changes after successive washes are checked with an Ives Tintometer or a Taylor Photometer as modified by Rhodes. No standard apparatus has been adopted as yet by the committee. Polesie (5) advocates the use of the step photometer for measuring brightness after consecutive washes. His article is very brief and no data are given.

Tate (7), in a recent article, brings forth a highly inadequate theory of detergency which may be completely disregarded. His postulates are thoroughly in error. Levitt (3) discusses the theories of detergency brought forward to date, namely, the Brownian movement theory, the lubricant theory, and the colloidal theory. Draves and Clarkson (1) discuss a new method for the evaluation of wetting agents which bears indirectly on the present subject. Lennox and Gilmore (2) present a large amount of data from plant tests concerning sudsing and rinsing time. Samples of wash liquor were centrifuged and the amount of insoluble dirt determined in this manner. This is the first work of this type on record and is on quite sound lines as far as insoluble dirt is concerned. Their conclusions are valuable to the industry in that they have determined the optimum times for the removal of solid dirt. Stain removal is not considered.

The main incentive to the present work was a paper by Rhodes and Brainard (6) in which it is claimed that a satisfactory method for the evaluation of the detergent efficiency of soaps has been worked out. It was, however, felt by the writer that some of the results might be rather misleading if considered as applicable to laundry practice. The method used by Rhodes and Brainard (6) was that of washing small samples of standardly soiled fabric in a miniature wash wheel (13.3 by 12.7 cm.) which was always driven in the one direction, usually at 80 r.p.m. The washing conditions were varied with respect to temperature, time, soap concentration, and rate of agitation. Inspection of the graphical results shown in their paper indicates immediately that the standardly soiled cloth was not sufficiently sensitive to small variations in the washing processes. The first illustration of this is in the comparison of runs at 20°, 40° and 60°C. The curves intersect at several places. A second illustration is presented in the runs at different rates of agitation. Here the 60 r.p.m. and 80 r.p.m. curves intersect and the final difference in brightness is of the order of 2% when compared with a baryta white standard.

The lack of sensitivity of the soiled fabric to washing is particularly apparent when the effect of the concentration of soap is investigated. Soap concentrations of 0.00, 0.01, 0.05, 0.10, 0.25, 0.50 and 1.00% were used. With concentrations of 0.05% and higher no appreciable difference in washing power was noted.

When the increase in brightness produced by a given number of washes was

plotted against the time of one wash by Rhodes and Brainard (6), very sharp maxima in brightness were obtained at the end of a 7.5-min. washing period. If longer periods were used the dirt was redeposited in the fabric up to the 30-min. region, after which the curve flattened out and became practically parallel to the time axis. The washing conditions for obtaining these results were as follows:—Temperature, 40°C.; soap concentration, 0.25%; rate of agitation, 80 r.p.m. No satisfactory explanation of these maxima are given and similar work by the writer of this paper affords no evidence that they exist. This feature will be discussed more fully in a later section.

As a conclusion to their work, Rhodes and Brainard (6) ran tests on five intrinsically different soaps, namely, commercial soap flakes, potash-coconut oil liquid soap, powdered olive castile soap, tallow soap, and potassium oleate. The tests did not indicate any marked differences in detergent power. This would hardly be expected, due to the lack of sensitivity of the soil. "Indices of Detergent Power" were calculated. The validity of the values obtained is questionable due to several assumptions which are made in the calculations and also to the very slight differences in the detergent power obtained experimentally. In the opinion of the writer the Rhodes and Brainard (6) method of evaluating the detergent power of washing liquors is a step in the right direction but the method has not been subjected to sufficient refinements. The present paper deals with refinements of this method and is preliminary to a paper dealing with the detergent efficiency of neutral and built soaps.

Experimental

Soiling

After considerable experimentation with different soils it was concluded that a modification of the soil advocated by the Detergents Committee of the American Oil Chemists' Society was the most satisfactory. The formula is as follows:—Carbon tetrachloride, 2000 cc.; Russian tallow, 3 gm.; Nujol, 10 gm.; lampblack, 2 gm.

In a standard soil it is essential that ingredients which are as nearly standard as possible be used throughout. The A.O.C. soil contained tallow and lubricating oil. Wide variations in chemical properties may occur in these products; hence in the present work Russian tallow and Nujol were substituted. The former contains less unsaturated material and will not become rancid as ordinary tallow will. Nujol, a medicinal oil, has constant chemical properties and is much easier to obtain than a uniform grade of lubricating oil. A large quantity of lampblack was obtained from one source and stored in order that this product, which exhibits large variations in particle size, might be as uniform as possible. The carbon tetrachloride used was redistilled.

In preparing the soil batch the Nujol and tallow were dissolved in the carbon tetrachloride, the lampblack was added and the whole was agitated at a fixed constant rate for 15 min.

Prior to soiling, the cotton sheeting (Wabasso No. 55, thread count 82 by 68) was desized by first washing it twice with the white work in a local power

laundry, followed by a 90-min. treatment in 2% Diastafor. The fabric was then rinsed 10 times in distilled water, ironed dry, conditioned in a thermostatically controlled oven at 80°C. for one hour, and then air conditioned in the laboratory overnight.

One of the main objections to the Rhodes and Brainard (6) soil was that it was not sufficiently sensitive to small changes in washing procedure. This may be attributed to a large extent to the mode of application. The fabric samples were passed by hand through a portion of the soil mix until the proper shade of grey was obtained, blotted between several thicknesses of cheesecloth, dried in an oven at 80°C. for one hour, and then air conditioned for 11 hr. In the opinion of the writer this does not sufficiently impregnate the fabric with the soil mix, particularly the insoluble material, the lampblack. Only a surface coating is obtained.

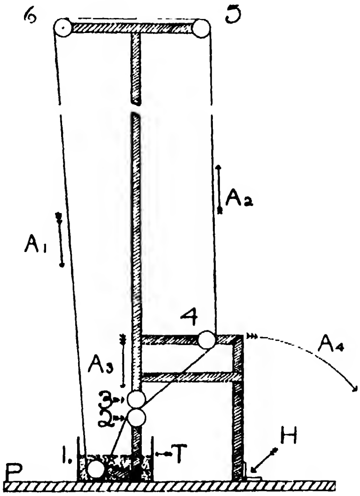

FIG. 1. *Soiling machine.*

The machine used in soiling the fabric in the present work is illustrated diagrammatically in Fig. 1. In reality this is a good quality household wringer set into a suitable framework. The rubber covered rollers (indicated by the numbers 2 and 3) of the wringer are operated by a chain drive from a motor and reducing gear not shown in the diagram. This insures a constant rate of drive. The whole mechanism is mounted on the platform P. The upper structure is hinged to the platform at H so that it may be tipped over in the direction of the arrow A_4 to facilitate the removal of the soil trough T. The machine accommodates a strip of cloth 14 ft. long and 9 in. wide. The cloth travels in the direction of the arrows A_1 and A_2 and in doing so passes under roller 1 in the soil bath, between rollers 2 and 3 where excess soil is wrung out and flows back into the trough T, under roller 4, over rollers 5 and 6, and then back to the soil bath. Each complete circuit of the cloth requires one minute and each sample is given ten passes through the soil bath. An equalizing pressure of 25 lb. is maintained on rollers 2 and 3 as indicated by the arrow A_3. This is accomplished by a free moving lever hinged to the upright at one end of the rollers approximately six inches above them, and passing horizontally to beyond the outer edge of the upright on the opposite side. From this projecting end a weight is suspended. A metal pin of 0.25 in. diameter connects the lever arm to a spring which rides on the axle ends of the upper roller 3. This takes care of any minute irregularities which may be present in the rollers. The above procedure produces a very evenly soiled sample with a reflection of 11.5% as compared with baryta white. Soiled strips can be duplicated with satisfactory regularity. This will be corroborated with experimental data in a later section.

Washing

The miniature wash wheel used in this work, illustrated in Figs. 2 and 3, is an improved model of that used by Rhodes and Brainard (6). The improvements introduced with this machine are the following:—(1) All metal parts coming in contact with the wash liquors are of Monel metal. (2) The machine reverses after a given number of revolutions, simulating the motion of a power laundry wash wheel. (3) The r.p.m. of the wash wheel may be widely varied as well as the number of revolutions before reversal.

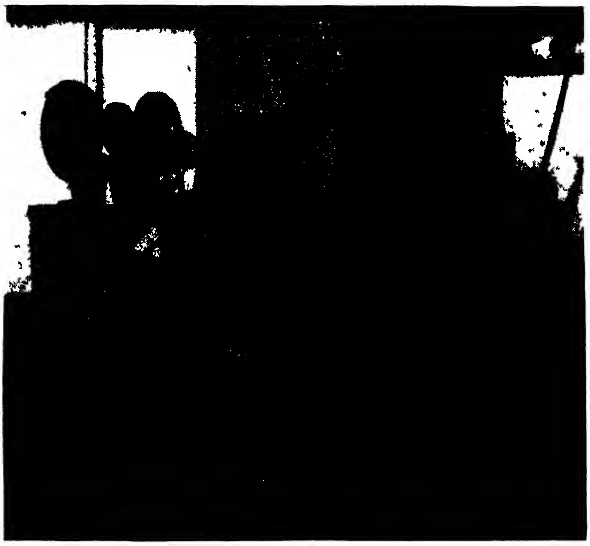

FIG. 2. *Miniature wash wheel.*

The machine consists of a glass cylinder of 11.5 cm. diameter and 16.5 cm. long. Inside the cylinder is a Monel metal framework carrying six bars of 1 cm. diameter which correspond to the ribs in a laundry wash wheel. The ends of the cylinder are closed by two Monel metal end plates containing countersunk rubber gaskets into which the glass cylinder fits. The end plates are connected outside the cylinder by six strong coil springs, thus forming a water-tight system. Through the centre of each end plate a Monel tube (1.2 cm. outside diameter) is fitted. These tubes are used for filling and draining the cylinder and at the same time act as trunnion bearings when the whole is mounted in the wooden housing illustrated in Fig. 2. A 150-watt light bulb supplies light and heat to the wheel and the temperature may be controlled to $\pm 1°C$. The bulb is situated directly below the wash wheel and is protected from odd drops of water by a shield of fine copper gauze.

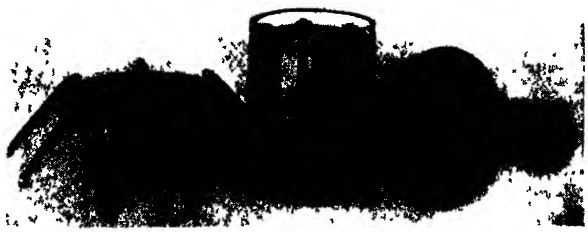

FIG. 3. *Wash wheel assembly dismantled.*

The driving mechanism, when the machine is driven constantly in one direction, consists of a motor and a reducing countershaft. When the machine is reversed after from one to three revolutions, the arrangement shown in Fig. 2 is used. A round leather belt is attached to a coil spring near the floor,

looped around the driving pulley on the wash wheel, and passes from there to the crank arm of the reducing gear. The wash wheel rotates in one direction until the crank becomes horizontal and then reverses for the same number of revolutions in the opposite direction. The number of revolutions before reversal may be altered by changing the length of the crank. The r.p.m. may be altered by supplying the motor and reducing gear with various sized pulleys.

To facilitate filling and draining the machine, the wooden housing is hinged at its base and may be tipped over on to the horizontal rests shown at the right. The lower end plate of the wash wheel is bevelled to the centre to insure complete draining. To insert and remove the fabric samples the upper end plate is removed.

The wash samples consist of a piece of standardly soiled cloth 10 by 20 cm. doubled, sewed up in bag form, and weighted with 100 gm. of glass beads of 3 mm. average diameter. Two of these bags are washed in 500 cc. of wash liquor in each operation.

Measurement of Whiteness

The criterion of the amount of washing which has taken place over a certain period is determined by measuring the brightness of the washed samples with a step photometer. A Zeiss Pulfrich photometer was used. This instrument is well described by Weltzien (9, pp. 46-53) and also briefly in the present connection by Morgan (4, p. 38).

Experimental Results

Testing Soil Batches

After soil batches were prepared as described in a previous section their washing properties were compared. A 0.25% neutral soap solution was used. This was prepared from a soap with the following analysis:—moisture, 2.01; total soap, 97.05; NaCl, 0.004; free alkali (Na_2O), 0.045; glycerol (by diff.), 0.89%: titer, 39.70°C. Washing and rinsing were carried out in the miniature wash wheel at 50°C., there being a 10-min. suds and two one-minute rinses in each operation. Each soil batch test consisted of two operations. The wash wheel was driven at 85 r.p.m. and reversed every 2.75 revolutions. All wash and rinse liquors were preheated to the desired temperature before introducing

TABLE I

RESULTS OBTAINED IN TESTING OF SOIL BATCHES

Wash No.	Batch number				
	1	2	3	4	5
	Percent brightness increase				
0	11 0	11 0	11 3	11 2	11 5
1	25 1	24 8	24 2	24 4	24 7
2	32 0	30 7	31 1	32 1	31 6

them into the wash wheel. Distilled water was used exclusively in all this work. Tests of this nature on five typical batches of soiled sheeting are included in Table I. It will be noted that the variations from one batch to another were comparatively small. Since each soiled strip was 14 ft. long, a large number of wash samples could be cut from each one. Comparisons of these samples gave identical results within the limit of experimental error.

Time Period Effect

The optimum length of time which a wash wheel should be operated in order to obtain maximum washing efficiency is a very important question. This has been investigated using washing periods of 2, 5, 7, 10, 20, 30, and 60 min. and temperatures of 25°, 50°, and 75°C. The data are presented numerically in Table II and graphically in Figs. 4, 5, and 6.

TABLE II
TIME PERIOD EFFECT DATA

No. of washes	Time period of one wash, min.						
	2	5	7	10	20	30	60
	Per cent brightness increase						
25° C.							
1	11.4	15 7	16.1	17 8	21.3	25.4	31.1
2	17.9	22.4	25.1	29.3	31.7	34.5	41.3
3	23.3	28 5	30.7	33.4	35.2	37.3	43.8
4	28 0	32.5	33.5	37.0	38.7	39.7	44.1
5	30.1	34 4	36.1	40.8	41.1	42.3	45.6
50° C.							
1	16.6	22.4	24.1	25.5	29.6	32.6	35.3
2	27.2	33.0	33.5	35.4	36.5	37.2	42.0
3	31.1	37.8	38.8	38.8	39 8	41.8	43.8
4	35.8	41.2	40.3	41.0	43.0	44.2	46.3
5	38.4	42.2	41.5	41.5	43.5	45.2	47.4
75° C.							
1	17.5	21.0	21.5	24.6	26.8	29.5	32.2
2	28.1	30.4	31.6	33.0	35.0	36.9	41.6
3	33.3	34.4	35.1	35.7	38.2	39.9	42.3
4	37.2	37.2	36.9	39.0	42.3	44.1	46.2
5	40.1	40.6	40.5	40.6	42.9	45.2	47.5

Each of the figures in the above tables was obtained by washing two samples of the standardly soiled fabric and taking the average of their respective increases in brightness. Each pair of samples was given five consecutive washes.

When total washing time is plotted against increase in brightness for each of the three temperatures, smooth curves are the result. This indicates uni-

formity in the soiling operation as well as in the washing operation. Only in one case do two curves intersect. This is in the 25°C. data where the 30-min. and 60-min. curves intersect in the 90-150-min. region. However, this region is relatively unimportant. The slopes of these curves would indicate that the optimum washing period lies in the 7-10-min. region. In practice, lightly

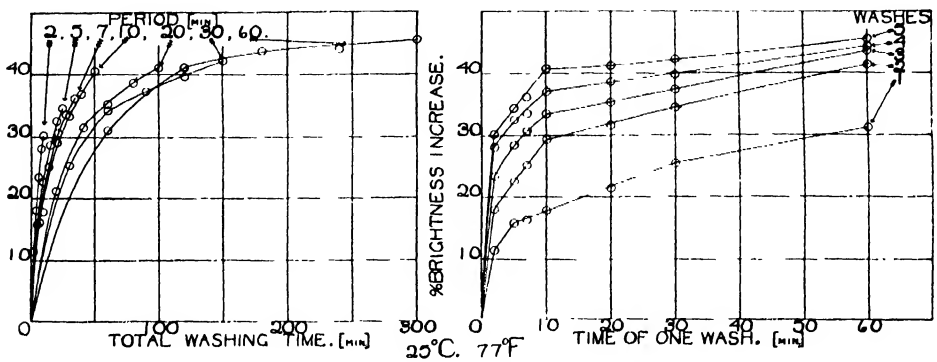

FIG. 4. *Time period effect, 25°C.*

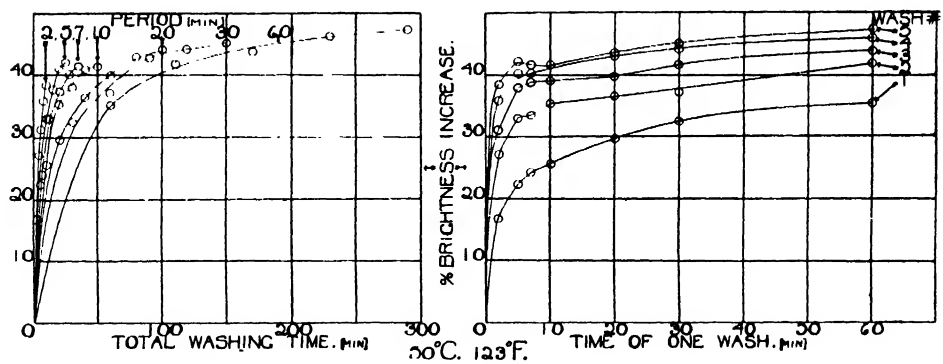

FIG. 5. *Time period effect, 50°C.*

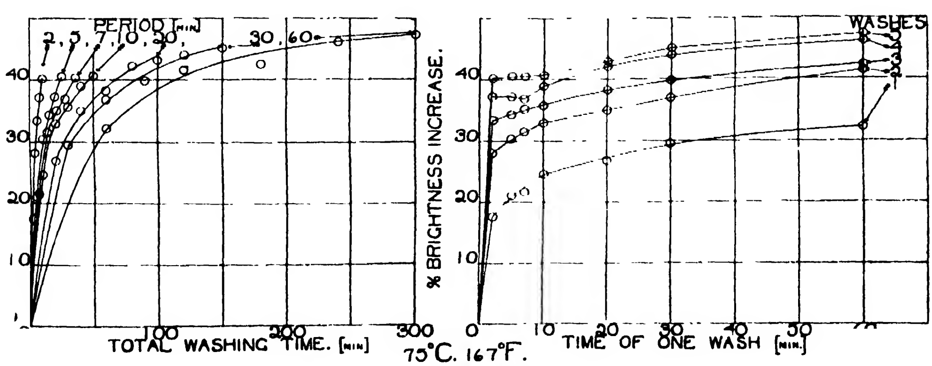

FIG. 6. *Time period effect, 75°C.*

soiled loads might be given a 7-min. treatment, and more heavily soiled loads a 10-min. treatment. If longer periods than 10 min. are used the efficiency decreases; shorter periods than 7 min. are very apt to be inefficient due to unstable emulsions or suspensions of the dirt being formed which would hinder it being rinsed free from the fabric.

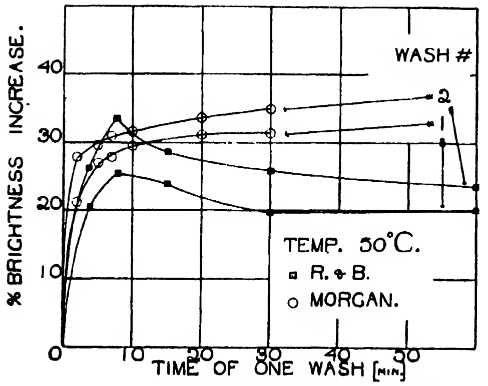

FIG. 7. *Rhodes and Brainard (6) and Morgan's data compared.*

When the time of one wash is plotted against increase in brightness, five curves are obtained, one for each of the five consecutive washes. This will show up very definitely any optimum time period of washing and the present data advocate 7-10 min. as stated above. Rhodes and Brainard (6), when plotting their data in this fashion, obtained sharp maxima at 7.5 min. It was thought by the writer that possibly the method of soiling had some bearing on this phenomenon. The Rhodes and Brainard (6) conditions were duplicated and the data obtained are compared with the Rhodes and Brainard (6) data in Table III and Fig. 7. After the second wash no maxima had appeared. Hence the experiment was discontinued. It has been impossible to give any logical explanation for the Rhodes and Brainard (6) maxima.

TABLE III

COMPARISON OF DATA OBTAINED BY RHODES AND BRAINARD WITH DATA OBTAINED IN THE PRESENT WORK

Rhodes and Brainard data						Data from present work						
	Time period of one wash, min.						Time period of one wash, min.					
	3.75	7.5	15	30	60		2	5	7	10	20	30
No. of washes	Per cent brightness increase					No. of washes	Per cent brightness increase					
1	20.7	25.2	23.2	19.8	19.9	1	21.2	27.0	27.8	29.5	31.2	31.3
2	26.8	33.0	28.5	25.8	23.6	2	27.8	29.5	31.0	31.7	33.6	35.1

It should be noted that in the Rhodes and Brainard (6) data there are only three points in the curves prior to 30 min. to define the maxima while in the present work there are five defining points. This helps materially to eliminate any discrepancies which might arise in this important region.

Table II contains the second set of data obtained for the time period effect. In the first set no maxima were obtained with a 7.5-min. washing period. The work was repeated to make sure that the phenomena did not exist. Hence it is felt that this point has been conclusively proved.

Effect of Temperature

In Fig. 8 total washing time is plotted against per cent brightness increase for 25°, 50°, and 75°C. data considering 10-min. washing periods only. It will be noted that the 75°C. curve falls between the 25°C. and 50°C. curves indicating that the greatest washing efficiency is obtained at 50°C. In order to establish this optimum temperature more rigorously, washes were conducted at 40°C. and 60°C. The complete set of data for all temperatures investigated is presented in Table IV and illustrated graphically in Fig. 9 where temperature is plotted against per cent brightness increase for the five consecutive washes.

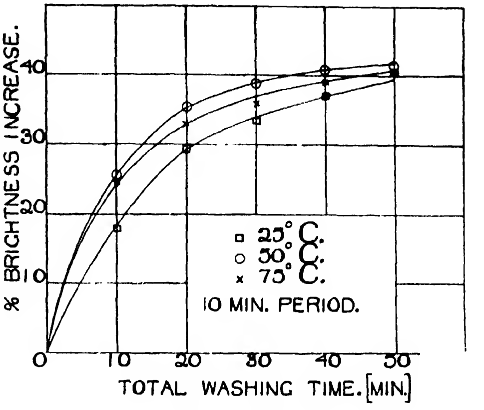

FIG. 8. *Effect of temperature; 25°, 50°, 75°C.*

In all washes except the first the maximum detergent efficiency appears at 50°C. This is of more theoretical than practical interest since, in laundry practice, the first suds operation is always performed at a low temperature in order that albuminous material may be removed. If higher temperatures were used, the albumen would be coagulated and its removal from the fabric would be rendered very difficult.

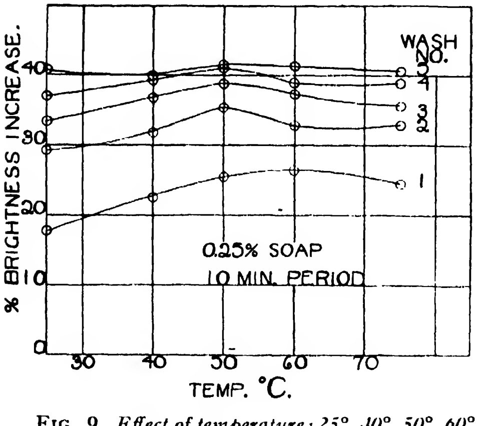

FIG. 9. *Effect of temperature; 25°, 40°, 50°, 60°, 75°C.*

In laundry practice it is recognized that temperature has a definite bearing on soil removal. The type of standard soil used by Rhodes and Brainard (6) was not sufficiently sensitive to define clearly the temperature coefficient of detergency. The soil used in this work does show up temperature effects but it is illogical to advocate definite plant temperatures for optimum detergent effects until full scale operations have been thoroughly investigated. These investigations will be carried out at a future date. The point which the writer wishes to stress at present is that the standard soil is sufficiently sensitive to indicate differences which do exist.

Effect of Soap Concentration

A further indication of the sensitivity of the standard soil used in the present work is furnished when the effect of soap concentration on detergency is investigated. Eight concentrations of soap were used ranging from 0.00 to

TABLE IV

EFFECT OF TEMPERATURE ON DETERGENT ACTION

No. of washes	Temperature, °C.				
	25	40	50	60	75
	Per cent brightness increase				
1	17.8	22 6	25.5	26.5	24.6
2	29.3	31.8	35.4	32.8	33.0
3	33.4	36 8	38.8	37.3	35.7
4	37 0	39 2	41.0	39.0	39.0
5	40 8	39.8	41 5	41 3	40.6

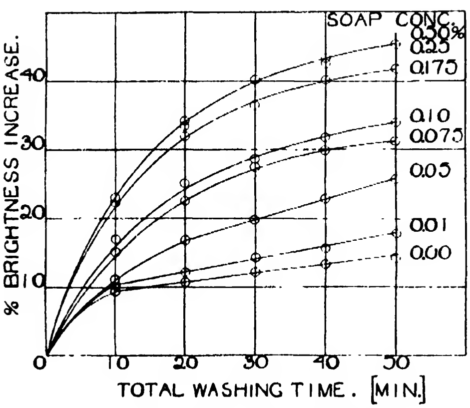

FIG. 10. *Effect of soap concentration.*

0.50% by weight of dry soap. The washing conditions were as follows:—temperature, 50°C.; r.p.m. 85; revolutions before reversal, 2.75; formula consisted of one 10-min. suds and two one-minute rinses. The results are contained in Table V and Fig. 10.

There is a gradual increase in detergent efficiency until a maximum is reached at 0.25%. The curves for 0.25% and 0.50% concentrations intersect in two places and fall almost on top of each other so that only one curve but two sets of points are shown in Fig. 10. In a similar experiment Rhodes and Brainard (6) showed only very slight increases in detergent efficiency above a concentration of 0.01%. For higher concentrations their curves lie in one region and intersect in several places due, doubtless, to the lack of sensitivity of their soil. In standard laundry practice a soap concentration in the

TABLE V

EFFECT OF SOAP CONCENTRATION ON DETERGENT ACTION

No. of washes	Soap concentration, % by weight of dry soap							
	0.0	0 01	0.05	0.075	0 10	0.175	0.25	0.50
	Per cent brightness increase							
1	9.5	10 3	11.1	17.0	15.7	22.5	23.0	22.4
2	10.7	12.1	16.8	22.5	25.1	32.0	33.5	34.2
3	12.2	14.4	19.9	27.6	28.6	36.6	40.3	40.2
4	13.3	15.7	22.9	29.8	31.7	40.3	43.5	42.5
5	14.2	17 7	25.6	31.3	34.0	41.8	45.4	45.5

region of 0.10% or slightly greater is maintained. From Fig. 10, considering only the present type of soil which is very difficult to remove, it is seen that this is quite an efficient concentration.

Effect of Reversing the Wash Wheel

Laundry wash wheels are driven two or three revolutions in one direction and then reversed. This prevents the load from rolling up into a ball-like mass and hindering the washing process. Also, at the time of reversal, there is vigorous agitation of the fabrics in the wash liquor. Experiments with the small wash wheel showed that, on the average, washing is 12.5% more efficient when the machine is reversed every 2.75 revolutions than when it is driven in one direction at the same rate. In these experiments the temperature was maintained at 50°C. and the rate of revolution at 85 r.p.m. The data are contained in Table VI and Fig. 11. Seven-, ten-, and twenty-minute washing periods were investigated.

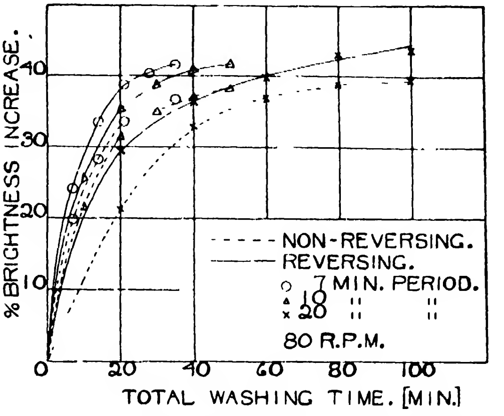

FIG. 11. *Washing with a reversing and non-reversing machine.*

TABLE VI

EFFECT OF REVERSING WASH WHEEL ON DETERGENT ACTION

	Time period of one wash, min.					
	7		10		20	
	a	b	a	b	a	b
No. of washes	Per cent brightness increase					
1	24.1	19.8	25.5	21.4	29.6	21.2
2	33.5	28.3	35.4	31.4	36.5	32.9
3	38.8	33.5	38.8	35.5	39 8	36 8
4	40.3	35.5	41.0	37.0	43.0	38.9
5	41.5	36 7	41.5	38.1	43.5	39.3

NOTE:—*a, reversing; b, non-reversing.*

Effect of Varying the R.P.M.

The experiments on the effect of varying the r.p.m. of the wash wheel were not conducted for the primary purpose of supplying data which could be applied directly to plant size machines, but to investigate the sensitivity of the standard soil to variations in the mechanical agitation during the washing process. This

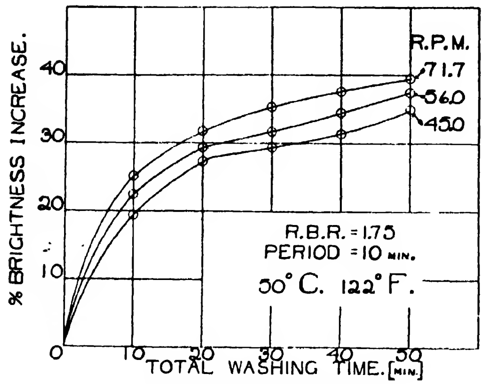

FIG. 12. *Varying r.p.m.*

factor is equally as important as the reaction of the soil to the supplies used in washing. Subsequent plant tests of a similar nature will provide data for the laundryowner.

It was found that the detergent efficiency increased with increasing rates of revolution. The temperature adopted was 50°C.; the period, 10 min.; the number of revolutions before reversal, 1.75; and the revolutions per minute, 45.0, 56.0, and 71.7. Table VII and Fig. 12 present the data obtained in these experiments.

TABLE VII

EFFECT OF VARYING R.P.M. ON DETERGENT ACTION

No. of washes	Revolutions per minute		
	71.7	56.0	45.0
	Per cent brightness increase		
1	25.2	22 4	19.3
2	31.9	29 4	27.4
3	35.3	31 6	29 3
4	37 6	34 5	31 2
5	39 6	37 5	35 1

Varying the Number of Revolutions Before Reversal

To investigate still further the effect of mechanical agitation on the standard soil the r.p.m. was maintained constant and the number of revolutions before reversal (R.B.R.) was changed from 1 to 1.75 to 2.75. The temperature was 50°C., the period 10 min. and the r.p.m. 70. Table VIII and Fig. 13 contain the results.

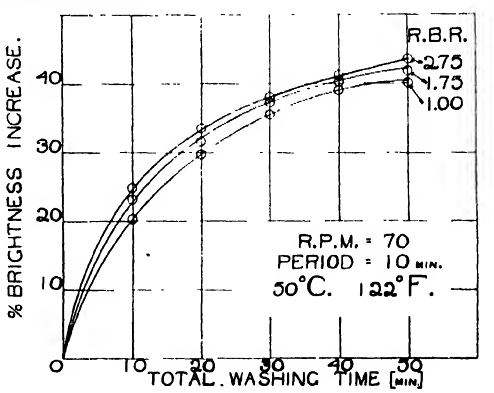

FIG. 13. *Varying the number of revolutions before reversal.*

With an R.P.M. of 70 and R.B.R. values of 1, 1.75 and 2.75, the number of reversals is 70, 40, and 25 respectively. One would expect that the greater the number of reversals, the greater would be the agitation, and that most efficient

washing would be obtained. However, this does not appear to be the case, since the best washing was obtained with 25 reversals, *i.e.*, 2.75 revolutions before reversal. This would indicate that there must be some sort of a balance between tumbling agitation and swirling agitation such as is obtained on the reversal. It would be a waste of time to go into this point more fully except on a plant scale.

TABLE VIII

EFFECT OF VARYING NUMBER OF REVOLUTIONS BEFORE REVERSAL ON DETERGENT ACTION

No. of washes	Revolutions before reversal		
	1	1 75	2.75
	Per cent brightness increase		
1	20 1	23.2	24.8
2	29.7	31.6	33 6
3	35 5	37.4	38.1
4	39 0	40.3	41 3
5	40.1	41.8	43.6

It may be pointed out that the data in Table VIII are not comparable with other data listed in this paper. The soil batch from which these samples were cut did not check up with soil batches used in other parts of this work. Since only intercomparisons were required, it was considered sufficiently accurate for this part of the work.

Conclusion

The data contained in this paper show quite conclusively that the Rhodes and Brainard (6) method of determining the detergent efficiency of soap has been improved upon considerably. The improvements may be summarized as follows:—

(1) The miniature wash wheel is constructed of non-corroding Monel metal. The ribs in the machine are of Monel metal and are not rubber covered.

(2) The driving mechanism is capable of wide variations with respect to speed and reversibility.

(3) The soiling procedure has been rendered uniform and consistent results are readily obtainable.

(4) The soil is sensitive to small differences in chemical treatment and mechanical agitation during washing.

With the sensitivity of the soil, established work is now under way in which neutral and built soaps are being compared with respect to their washing power. The physical properties of their solutions are also under investigation. The results will be published in this journal upon completion.

References

1. Draves, C. Z. and Clarkson, R. G. Am. Dyestuff Reporter, 20: 201-208. 1931.
2. Lennox, C. E. and Gilmore, B. H. Special Bulletin, Laundryowners National Association. March, 1929.
3. Levitt, B. Am. Dyestuff Reporter, 20: 641-642. 1931.
4. Morgan, O.M. Laundry and Dry Cleaning Journal of Canada, 11: 8-11, 34, 38, 41. 1931.
5. Polesie, L. Melliand Textilberichte (Eng. ed.) 12: 127. 1931.
6. Rhodes, F. H. and Brainard, S. W. Ind. Eng. Chem. 21: 60-68. 1929.
7 Tate, G. S. Soap. 7: 23-26. 1931.
8. Vail, J. G. Oil and Fat Industries, 8: 63-64. 1931.
9. Weltzien, W. Chemische und physikalische technologie der Kunstseiden Akademische Veilagsgesellschaft M.B.H. Leipzig. 1930.

OIL DAMAGE TO COTTON TENTING MATERIALS[1]

By O. M. Morgan[2]

Abstract

The effect of weathering on white and brown (Mineral Khaki) cotton duck tenting materials treated with a variety of oils has been investigated. Vegetable oils have the greatest deteriorating action. Cottonseed oil has been shown to produce the greatest weakening effect, giving tensile strength losses of 73% during a 240-day exposure. Mineral oil exerts only a moderate damaging effect. It is possible that the tensile strength losses are proportional to the iodine values but this has not been definitely established. Mineral Khaki has a very definite weather-proofing and oil resisting action when impregnated in cotton.

Several months ago two damaged tent tops were submitted, by the Department of National Defence, to this laboratory for inspection. The damage appeared to have been caused by oils, as the fabric was quite rotten where oil spots occurred. A search of the literature revealed very little helpful information. Phair and Lukash (1) in a recent paper discuss oil damage to cottons in laundering after exposure to sunlight. Losses of as much as 49% in tensile strength were experienced with cottonseed oil after sun treatment for 48 hr. and then washing. In the dark after 14 days the loss was 10%.

The amount of oil added to each sample was not stated. The treatment, however, consisted of impregnating the samples with oil, exposing them in the light and also in the dark, and then making tensile strength tests before and after washing. The authors conclude that the damage may be attributed to two causes. The first is the fact that the oils lubricate the fabric. The second is their power to absorb oxygen, or oxidize. Drying oils, possessing this property to a marked extent, oxidize and weaken the cotton fibres. Tests for oxycellulose are readily obtainable after the above treatment. Light accelerates the oxidation.

No data on heavy fabrics such as duck were available.

Experimental

New samples of white and brown duck were obtained, treated with a variety of oils, stretched on a frame, and exposed to the weather on the roof of the laboratory from April 15 to December 11, 1931. The frame was tilted considerably to give the samples a southern exposure. After 125 days' exposure each sample was divided and one-half was brought into the laboratory for tensile strength tests.

The characteristics of the new samples of duck are contained in Table I.

It may be seen that the white and the brown samples are identical as far as weave is concerned. The brown duck had been treated with "Mineral Khaki" (2, p. 897). It was found to have an ash content of 1.75% containing iron-chromium salts and a trace of silica. This is a fast dye and a good weather-proofing agent.

The chemical characteristics of the oils used are given in Table II.

[1] *Manuscript received January 29, 1932.*
Contribution from the National Research Laboratories, Ottawa, Canada.
[2] *Assistant Research Chemist, National Research Laboratories, Ottawa, Canada.*

TABLE I

PROPERTIES OF DUCK SAMPLES USED

Type of duck	Tensile strength, lb.	Thread count		Ply of yarn		Chemical treatment
		Warp	Filling	Warp	Filling	
White	150.4	50	30	3	4	—
Brown	165.4	50	30	3	4	Mineral Khaki

TABLE II

PROPERTIES OF OILS USED

Oil	Type	Acid value	Iodine value
Mineral lubricating	Non-drying	0.33	12.7
Linseed	Drying	2.46	177
Cottonseed	Semi-drying	0.44	109
Castor	Non-drying	1.00	85

Each sample of duck, 5 by 24 in., was sprayed with 20 gm. of oil dissolved in 25 cc. of ether to facilitate spreading.

Tensile strength tests were made using a Suter tensile strength machine with 1-in. jaws, a throw of 12 in. per min., and accommodating a sample 3 in. long. Table III shows the tensile strength losses after oil treatment and weathering.

TABLE III

EFFECT OF EXPOSURE AFTER TREATMENT WITH OILS

Type of duck	Exposure, days	Per cent tensile strength losses with:—				
		No oil	Mineral oil	Linseed oil	Cottonseed oil	Castor oil
White	125	4.2	14.8	59.5	68.8	50.6
	240	9.1	22.4	66.7	73.0	57.1
Brown	125	0.7	10.7	26.3	29.2	12.8
	240	1.2	19.6	27.3	34.1	13.9

In order to determine the loss in tensile strength due to the lubrication of the fabric by the oil a similar set of oiled samples were prepared. These were allowed to stand for 24 hr. after spraying in order that the oil might soak into the fabric. Losses in strength were very similar for all the oils used. The white duck showed an average loss of 8.9% and the brown duck 6.3%.

Discussion

Inspection of Table III indicates that the amount of damage by the oils falls in the following order: cottonseed, linseed, castor, and mineral lubricating

oil. This order holds true for both the white and the brown duck. Hence, it may be concluded that vegetable oils are more damaging to cotton materials than mineral oils.

In the case of white duck, with the exception of linseed oil, the tensile strength losses are proportional to the iodine values of the oils. Linseed, since it is a drying oil and hardens on the fabric, may supply a certain amount of protection against weathering after the hardening is complete. The losses in strength suffered by the brown duck are not proportional to the iodine values of the oils.

The apparent relationship between the tensile strength loss of the white duck and the iodine values of the oils with which it was treated may be merely fortuitous. The relationship may, however, be a causal one. Further work would be required to decide the point definitely.

Duck treated with mineral khaki presents the following valuable characteristics—

(1) In weathering tests where no oil is present over a 240-day period its loss in tensile strength is 1.21% as compared with 9.11% for white duck.

(2) In weathering tests where vegetable oils are present its total tensile strength loss is 40.8% lower on the average than that of white duck. When mineral oil is used it is 2.8% lower.

(3) Oil does not penetrate nor lubricate mineral khaki duck to the same extent that it does white duck. The lubrication loss in tensile strength for the former is 6.3% and for the latter 8.9%. It is impossible to differentiate between losses due to weathering and lubrication after the samples have been exposed for some time, since the lubricating properties of the oils change to a marked extent. This is particularly true in the case of drying and semi-drying oils.

It is strongly recommended that tents, tenting materials, sail cloth, or cotton fabrics of any kind should not be stored or transported in close proximity to oil drums, oily machinery, or the like. Especial care should be taken to avoid their contact with vegetable oils. Also, oil spots should be removed immediately upon detection by using organic solvents such as ether, chloroform or carbon tetrachloride.

Acknowledgments

The writer wishes to thank the Department of National Defence, Ottawa, for supplying the new samples of tenting materials used in this work, and Mr. C. W. Davis of this laboratory for analyzing the sample of brown duck.

References

1. PHAIR, R. A. and LUKASH, J. G., Laundry Age 10: No. 12: 30-32. 1931.
2. MILLS, L. J. (Ed) Textile Educator, v. 2. I. PITMAN and SONS. 1927.

THE COMPARISON OF GASEOUS DENSITIES BY THE METHOD OF BALANCING COLUMNS[1]

By Arthur H. Snell[2] and A. Norman Shaw[3]

Abstract

An application of the principle of balancing columns to the comparison of gaseous densities at any ordinary pressure is described. The precision of a given apparatus depends upon the heights of the columns, on the sensitivity of the pressure gauge, and on the extent of uncontrolled fluctuations in temperature. Without a thermostatic control and employing a simple modified form of the Toepler micromanometer with columns 12 m. in height, a sensitivity of 10^{-7} gm. per cc. is obtained. The apparatus and the technique are explained in detail, with a discussion on the elimination of errors. An application of the method is made in the field of hygrometry, and its usefulness in the measurement of air-gas ratios is also explained and recommended. An advance in precision is obtained, primarily by the use of an improved form of Toepler micromanometer.

Introduction

If two gases of different densities are placed respectively in two closed vertical columns, the pressure being kept the same at the tops, then the difference in density of the gases will produce a difference in the hydrostatic pressure at the bases of the columns; or, conversely, it can be stated that the reading of a suitable pressure gauge connected between the bases of two such columns is a measure of the difference in density between the gases which they contain. This is the principle involved in the "balancing columns" method of comparing the densities of gases or gaseous mixtures.

Romberg and Blau (1) applied the method to wet and dry air, in developing an absolute hygrometer based on the determination by this method of the difference in density between the sample to be tested and a standard sample of saturated air. The same principle had also been tried roughly by one of the present writers, using an inclined gauge which gave results of too low an accuracy to be of practical interest. The present investigation was originally intended to be a development of this method, with the greater precision which might be expected from higher columns, the use of a standard column of dry air instead of saturated air, and improved methods of measuring the small pressure difference which is developed at the bottom. It was soon found, however, that the usefulness of the method is by no means confined to hygrometry, and that its possibilities as a means of comparing gaseous densities in general have not been fully realized; the former is only one field of many in

[1] *Manuscript received January 29, 1931.*
Contribution from the Department of Physics, McGill University, Montreal, Canada, with financial assistance from the National Research Council of Canada.

[2] *Graduate student in Physics, McGill University, and holder of a bursary under the National Research Council of Canada.*
[3] *Professor of Physics, McGill University.*

Note by A. N. Shaw: *A preliminary account was presented verbally at the meeting of the Royal Society of Canada in Toronto, in May, 1931. This work was part of an investigation on hygrometrical problems carried out under a grant from the National Research Council, by Mr. A. H. Snell, acting as my assistant. Some promising initial tests had been made by Dr. H. W. Harkness, Acadia University, while acting in the same capacity and the scheme had been considered theoretically, but apart from general supervision and advice the whole of this investigation on the method of balancing columns as reported in this paper has been developed by Mr. A. H. Snell.*

which the method may be useful. At the same time, however, it should be pointed out that to use the balancing columns as a hygrometer is to subject it to a performance test of a most rigorous nature.

In the course of the investigation considerable experience was gained and some advances were made in the use of the Toepler tilting micromanometer. The simplicity and low cost of the instrument, coupled with its great sensitivity, recommend it for many purposes now served by more elaborate and expensive micromanometers. It was accordingly considered useful to include in this paper some remarks on the performance of the instrument, together with an account of some improvements in its design.

Theory of the Method of Balancing Columns

In a vertical column of gas the contribution to the pressure at its base which is made by a small layer of gas at a distance x from the top, and having a thickness dx, is given by

$$dp = g\rho dx. \tag{1}$$

The relation between p and ρ may be obtained from the equation $pv = RT$, or, if greater accuracy is desired, from one of the more elaborate equations of state. Let us, for a close approximation, take the relation given by Van der Waals' equation in the form

$$pv = RT + Bp,$$

where $B = b - \frac{a}{RT}$, a and b being the familiar Van der Waals' constants. This gives

$$\rho = \frac{\frac{pM}{RT}}{1 + \frac{Bp}{RT}}, \tag{2}$$

where M represents the molecular weight of the gas. Substitution of this value for ρ in Equation (1) followed by integration gives

$$RT \log p_B + Bp_B = gMh + RT \log p_A + Bp_A,$$

where p_A and p_B refer respectively to the pressures at the top and the base of a column of height h. This in turn can be written in the form

$$\frac{p_B - p_A}{p_A} - \frac{1}{2}\left(\frac{p_B - p_A}{p_A}\right)^2 + \frac{B}{RT}(p_B - p_A) = \frac{gMh}{RT},$$

where terms in the third and higher powers of $(p_B - p_A)/p_A$ have been neglected.

Further rearrangement and the elimination of M by virtue of Equation (2) leads readily to the form

$$p_B - p_A = \frac{gh\rho_A}{1 - \left\{\frac{1}{2}\frac{p_B - p_A}{p_A} \Big/ \left(1 + \frac{Bp_A}{RT}\right)\right\}}$$

where ρ_A represents the density of the gas at the pressure p_A. Now the terms Bp_A/RT and $(p_B - p_A)/p_A$ are both so small that their products and higher powers may be found on substitution in the present problem to be negligible. It is therefore evident that the expression reduces to

$$p_B - p_A = gh\rho_A\left(1 + \frac{1}{2}\frac{p_B - p_A}{p_A}\right) = gh\rho_A\left(1 + \frac{1}{2}\frac{gh\rho_A}{p_A}\right).$$

Since B does not survive in this expression it is now apparent that the simple equation of state, $pv = RT$, is adequate for our present purpose.

Similarly for a column of the same height but containing a different gas,

$$p'_B - p'_A = gh\rho'_A\left(1 + \tfrac{1}{2}\,\frac{gh\rho'_A}{p'_A}\right).$$

Now if the columns are connected across the top, $p_A = p'_A$, and consequently the difference in the pressures at their bases (*i.e.*, the pressure registered by the pressure gauge) is given by

$$\Delta p = p_B - p'_B = gh(\rho_A - \rho'_A)\left(1 + \tfrac{1}{2}gh\,\frac{\rho_A + \rho'_A}{p_A}\right) \tag{3}$$

(It is convenient to use the symbol Δp to represent the small difference in pressure at the bases of the columns).

This final expression may be regarded as the general operating formula for the balancing columns. In the great majority of cases the last term on the right-hand side will be found to be negligible. Only with heavy gases in long columns will it have an appreciable effect*. Thus for general use the formula usually reduces to

$$\Delta p = gh(\rho_A - \rho'_A),$$

or simply

$$\Delta p = gh(\rho - \rho'), \tag{4}$$

where ρ and ρ' represent the respective densities of the two gases at the pressure and temperature prevailing at the time of the experiment.

Discussion of Temperature Effects

To avoid difficult measurements and calculations it is obvious that the two columns should be kept as nearly as possible at the same temperature. In order to do this it was found most convenient to place one column inside the other. In practice three possible types of temperature variations yet remain in this arrangement, which may affect the reading of the pressure gauge at the bottom of the columns. They are as follows: (i) simultaneous equal changes in temperature affecting both columns; (ii) a variation in temperature from point to point up each column, such variation being equal in the two columns because of their close contact; (iii) a small fluctuating difference in temperature between the two columns.

Of these we might expect each of (i) and (ii) to balance out on the two sides of the gauge, and therefore have no effect upon its reading. This is obviously true of (i), but it is necessary to examine (ii) and (iii) in some detail.

In case (ii) let us suppose that there is the same linear temperature gradient up each column. This gives a rough idea of what to expect in practice since the temperature gradients which are apt to be encountered will be nearly equivalent to linear along portions of the columns. Let the temperature at the top of the columns be T_A, and at their bases, T_B. Then, if G signifies the temperature gradient, we have $G = (T_B - T_A)/h$.

**E.g., with columns 12 m. in height, filled with bromine and xenon respectively, the inclusion of this term would produce a change of about 0 6% in the value of Δp.*

It is apparent from Equation (1) that at a distance x from the top

$$dp = \frac{pM}{R(T_A + Gx)} gdx.$$

On integrating from top to bottom and applying to the case of two columns as before, we get

$$\Delta p = \frac{p_A gh \log (T_B/T_A)}{R\,(T_B - T_A)} (M - M') = gh\,(\rho - \rho') \left(1 - \tfrac{1}{2}\frac{T_B - T_A}{T_A}\right).$$

Thus the effect of an equal and uniform temperature gradient up both tubes is merely to multiply the Δp deduced on the assumption of a zero temperature gradient by the factor

$$\left(1 - \tfrac{1}{2}\frac{T_B - T_A}{T_A}\right). \tag{5}$$

It can be seen from this that a difference in temperature of 6° C. between the top and the bottom of the columns would be required to alter the value of Δp by 1 part in 100. A difference in temperature as large as this would rarely be encountered in practice.

In case (iii) there must be free intercommunication between the columns at the top, or otherwise an exceedingly slight variation in temperature (say one ten-thousandth of a degree) will be sufficient to disturb the pressure gauge sufficiently to make the reading hopelessly inaccurate*.

Let us suppose that one column is placed inside the other, and that both are initially at the temperature T. The temperature of the outer column is then raised to T', thus introducing a temperature difference of $T' - T$. Let V_0 = volume of outer column; V_i = volume of inner column; A = area of cross section of the annular outer column; a = area of cross section of inner column; ρ_0 = initial density of gas in outer column; ρ_0' = final density of gas in outer column; ρ_i = initial density of gas in inner column; ρ_i' = density of original gas in inner column if imagined to occupy $V_i - v$; v = volume of gas of density ρ_0 passing from the outer to the inner column through the connecting tube at the top.

Then after the temperature change has taken place the outer column will be filled with gas of density ρ_0'; while the inner column will contain a mixture which will be equivalent to a height $(h - v/a)$ of gas of density ρ_i', and a height v/a of gas of density ρ_0. Thus the change in the pressure difference between the bases of the columns will be given by

$$\delta\,(\Delta p) = gh\,(\rho_i - \rho_0) - \left\{ g\left(h - \frac{v}{a}\right)\rho_i' + g\rho_0\frac{v}{a} - g\rho_0' h \right\}.$$

Noting that $(V_i - v)\rho_i' = V_i\rho_i$, that $(V_0 + v)\rho_0' = V_0\rho_0$, and also that $v = V_0 V_i (T' - T)/T(V_0 + V_i)$ approximately, it can be shown that the above expression reduces to

$$\delta\,(\Delta p) = gh\rho_0 \frac{T' - T}{T}. \tag{6}$$

It will be noted that this is also very nearly the change in the gauge reading

*It was, in fact, observed that when the columns were joined at the top by a capillary tube 1 mm. n bore and 4 cm. long, the fluctuations observed by the pressure gauge made accurate reading impossible.

which would be produced if the gas in the outer column had been allowed to expand into the air instead of into the inner column. It is important to notice that the effect of the volume v of gas of one density being mixed with gas of the other density is itself negligible in the case under consideration. The transfer of the volume v exerts its influence only in that it permits a relative change in density in the columns when a temperature difference is maintained.

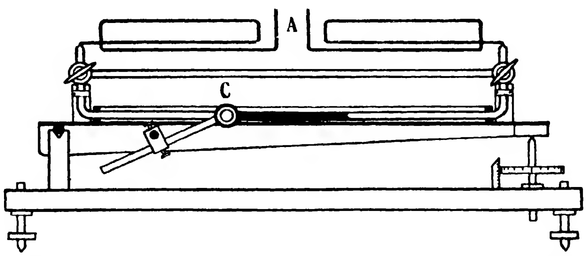

FIG. 1. *The Toepler micromanometer as modified for use with the balancing columns. The fine tubes lead from A to the bases of the columns. B is the tilting screw, and C is the microscope used for observing the meniscus of the manometer liquid (shown black).*

To obtain an idea of the magnitude of these effects suppose that the outer column of an apparatus having the dimensions of that described below is raised 0.01° C. above the inner column. Then, if both columns were originally at 20° C., it is found that $v = 0.03$ cc. Also if the outer column were filled with dry air $\delta(\Delta p) = 0.05$ dynes per cm^2., which is so small as to approach the limit of accuracy of the reading of the Toepler gauge. It is thus apparent that to be used to its full advantage an apparatus of these dimensions should be designed to keep the average differential temperature fluctuations between the columns within the bounds of 0.01° C. If this is impossible the experimenter must be content with diminished accuracy, but it would require fluctuations of about 0.3° C. to produce an error of 0.1% in ρ_i/ρ_o.

The obvious way to eliminate all disturbing temperature effects is to enclose the columns in a thermostat. This would, however, involve an elaborate and usually unnecessary addition to the technique. In measurements in which an accurate knowledge of the temperature of the columns is not required (*e.g.*, in the balancing columns hygrometer and in the measurement of air-gas ratios) one of the main advantages of the method lies precisely in the fact that high precision can be obtained without sacrificing simplicity in the apparatus. It is apparent that in any given case the constancy of repetition of the micromanometer readings will indicate whether or not the temperature control is adequate.

Discussion of Diffusion Effects

During any given determination the connecting tube at the top of the columns is open, and a small amount of diffusion must necessarily take place in both directions which, after a long time, will alter the difference in pressure at the bottom. A simple calculation shows that during the average time taken to make an observation with the micromanometer gauge the resulting correction will be negligible. It can be shown that this correction is given approximately by multiplying the right-hand side of Equation (4) by the factor

$$\left\{1 - \frac{Dat}{l}\left(\frac{1}{V_1} + \frac{1}{V_2}\right)\right\}, \tag{7}$$

where D is the coefficient of the diffusion for the two gases at the temperature of the experiment; a is the area of cross section of the connecting tube, and l is its length; V_1, V_2 are the volumes of the two columns; and t is the time in seconds during which diffusion takes place. In columns of the dimensions used in the present tests, joined by a connecting tube 10 cm. long and 4 mm. in bore, this factor would have the value 0.99973 in the case of diffusion taking place for one minute between columns containing carbon dioxide and air respectively. At this rate it would take about 40 min. for diffusion effects to produce a 1% change in the gauge reading. For other gases the value of D may be slightly greater, but it appears that with the given columns diffusion effects will be entirely negligible unless the readings cover an extremely protracted length of time.

The Use of the Balancing Columns as a Hygrometer

The difference in density which exists between dry air and damp air suggests the use of the columns as an absolute hygrometer. The sample of air which is to be tested can be placed in one column and balanced against a standard column of either dry air or saturated air. The use of a column of dry air is preferable because saturated air may lead to difficulties, as described below.

Dry and Damp Columns

For the present purpose it is adequate to assume for the density of the damp air the usual expression $\rho = 1.293 \times 10^{-3} \times 273\ (P - 0.378f)/760T$, where P denotes the barometric pressure in mm. of mercury, f the partial pressure exerted by the water vapor (also in mm. of mercury), and T the absolute temperature. Since the density of dry air is given by $1.293 \times 10^{-3} \times 273P/760T$, we have from Equation (4),

$$\Delta p = gh \times 1.293 \times 10^{-3} \times 273 \times 0.378f/760T,$$

whence $$f = 760T\Delta p/(gh \times 1.293 \times 10^{-3} \times 273 \times 0.378),$$

and $$H = 100 \times 760T\Delta p/(gh \times 1.293 \times 10^{-3} \times 273 \times 0.378F),$$

where H denotes the percentage relative humidity of the damp air, and F the maximum vapor pressure of water in millimetres of mercury at the temperature T. This may be written in the form

$$H = CT\Delta p/F \tag{8}$$

where C is a constant for the columns used. This may be regarded as the operating formula for the balancing columns hygrometer using a standard column of dry air.

Variations in the carbon dioxide or oxygen content of the air may be sufficient to cause changes in density which, if attributed solely to water vapor, might in extreme cases cause errors of several per cent of relative humidity in the measurements given by the columns. This possibility is eliminated in practice by drawing a new sample of dry air into the standard column whenever a new sample of damp air of the same kind is introduced into the other column.

Saturated and Damp Columns

If the air sample of unknown humidity is balanced against a standard column

of saturated air instead of dry air, reasoning similar to that given above leads to the expression

$$\Delta p = gh \times 1.293 \times 10^{-3} \times 273 \times 0.378(F-f)/760T \qquad (9)$$

This, however, assumes a pressure gradient in the vapor part of the column. If the walls of the column are wet we might expect that no such gradient would exist. On calculating the value of Δp from this point of view we get

$$\Delta p = gh \times 1.293 \times 10^{-3} \times 273(F-0.378f)/760T \qquad (10)$$

—a value considerably larger than the foregoing.

There is a small variation in the saturated vapor pressure F of a liquid with the external pressure p given by $dF/dp = V_1/V_2$ where V_1 is the volume of unit mass of the liquid, and V_2 that of unit mass of its vapor. Calculation shows that this is negligible in the present case.

A number of readings were taken using a column of saturated air which had been prepared by pouring a small quantity of water down one of the tubes. The values of Δp obtained were intermediate between those expected according to Equations (9) and (10), but were grouped chiefly about the former. Temperature effects in the saturated column made the readings exceedingly variable. It is clear that in the vicinity of the state of saturation temperature variations will have an exceptionally large effect, not covered by the reasoning already developed for unsaturated or dry gas samples.

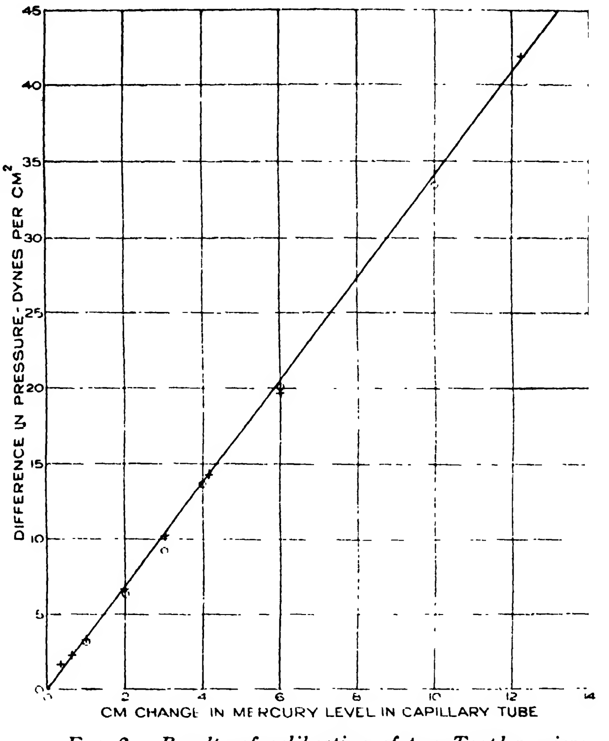

FIG. 2. *Results of calibration of two Toepler micromanometers. The straight line gives the theoretical relationship between the changes of level of the mercury in the capillary tube and the pressure changes thereby produced. The points* o *give the experimental values given by one gauge, using xylol as a manometer liquid; and the points* + *those given by the second gauge, using normal butyl phthalate.*

Application to the Measurement of Gaseous Ratios

The balancing columns may be used to determine the proportions by volume of the constituents in a mixture of two gases.

Let ρ_1', ρ_2' be the densities of the two constituent gases in the mixture, and let ρ_1, ρ_2 be their densities if each is reduced to the pressure of the mixture,

the temperature being unaltered. Under these conditions let their volumes be v_1 and v_2 respectively. Let ρ be the density of the mixture, and let r be the percentage by volume of the first constituent in the mixture, *i.e.*, $r = 100\, v_1/V$ where V is the volume of the mixture. Then it is apparent that

$$\rho = \rho_1' + \rho_2' = (v_1\rho_1 + v_2\rho_2)/V = \left\{r\rho_1 + (100 - r)\rho_2\right\}/100,$$

and therefore

$$r = 100(\rho - \rho_2)/(\rho_1 - \rho_2). \tag{11}$$

Now suppose that we have one column containing the second gas in its pure state. If we fill the second column in turn with the mixture and with the first constituent, we will obtain values for $(\Delta p)_1$ and $(\Delta p)_2$ where,

$$(\Delta p)_1 = gh(\rho - \rho_2) \quad \text{and} \quad (\Delta p)_2 = gh(\rho_1 - \rho_2).$$

Comparison of these results with Equation (11) shows that

$$r = 100(\Delta p)_1/(\Delta p)_2 = 100\, n_1/n_2, \tag{12}$$

where n_1 and n_2 represent the number of divisions of the tilting screw of the Toepler micromanometer required to compensate for the pressure differences $(\Delta p)_1$ and $(\Delta p)_2$ respectively (see Equation (13)).

Aside from its simplicity the great advantage of this method is that it is independent of the composition of either constituent of the mixture. This consideration is of value in testing such mixtures as air and coal-gas; *e.g.*, in rating commercial gas heating appliances. In such tests it is required to measure "air-gas ratios"—*i.e.*, the proportions in which air and gas are mixed before burning. Other devices have been improved recently for this purpose (2), but their operation and accuracy may be limited by the variability of the composition of the coal gas.

When the balancing columns method is applied to measurements of this type, it is most convenient to fill the standard column with air, and to balance against it a sample of the air-gas mixture and then a standard sample of the coal gas. With a micromanometer reading to 0.1 dyne per sq. cm. the columns need only be about one metre in height in order to measure r with an accuracy of within 0.1% of gas. Such precision is itself an improvement upon other instruments now in use, but if a simple compact instrument is not essential the use of the more delicate micromanometer with longer columns should easily improve the accuracy tenfold or more, temperature effects being the ultimate limiting factors.

Experimental

The Micromanometer

The tilting micromanometer devised by Toepler is particularly adapted for use with the balancing columns on account of its sensitivity and simplicity of construction. It consists essentially of a manometer tube in the shape of a very flat V mounted on a tilting table. One of the menisci is observed through a microscope fitted with a cross-hair. It is best used as a null instrument.

If the micrometer tilting screw must be turned through n divisions to compensate for the shift due to a pressure difference Δp at the ends of the tube,

and if φ is the angle of tilt corresponding to one of these divisions, then the value of Δp is given by

$$\Delta p = g\rho n\varphi\lambda, \tag{13}$$

where ρ is the density of the liquid and λ the horizontal distance between menisci (3). This expression disregards meniscus corrections. They are in general negligible in work with the balancing columns, for the pressure differences to be measured are usually small, and with a convenient value of λ (*e.g.*, 10 to 20 cm.) the angles of tilt involved in measurements are not large enough to introduce them to an appreciable extent.

For work with the balancing columns the original Toepler gauge was modified to the form shown in Fig. 1. The manometer tube is shunted through two three-way stopcocks by a second tube. This arrangement is convenient for setting the gauge to its zero position; it also increases the rigidity of the instrument. The fine, looped tubes which connect the ends of the manometer tube to the bases of the columns are made by drawing out a piece of 1-cm. glass tubing to arm's length, and bending it to the required shape in a yellow flame. Wax joints connect them to the columns and to the gauge. Their purpose is to eliminate distortion or strains in the manometer tube when the latter is tilted. Without these precautions and modifications, appreciable errors in the readings were produced on tilting.

Following Toepler, xylol was used as a manometer liquid. It has a slight disadvantage in that its vapor attacks stopcock grease. Ordinary kerosene also proved to be satisfactory. Normal butyl phthalate was tried because of its low vapor pressure, but it was found too viscous to make quick reading possible.

A sensitivity of better than 0.03 dynes per sq. cm. was obtained with this micromanometer.

Calibration of Micromanometer

A preliminary arrangement of the balancing columns apparatus gave results which were very irregular (due probably to distortion in the then unimproved gauge), and at the time it was thought that the trouble might arise from some unforeseen errors in the readings given by the micromanometer. To test this point it was decided to give the instrument an independent calibration. This was done by connecting it between two gas reservoirs one litre in capacity, and compressing the gas in one reservoir by from 1 to 40 parts in a million by adjusting the level of a thread of mercury in a capillary tube connected to that volume. To decrease the inevitable temperature effects as much as possible two Dewar flasks were used as reservoirs, and they were placed side by side in a water bath. The effect of temperature changes in the connecting tubes was reduced to a minimum by using tubes 0.5 mm. in bore, and by lagging all exposed parts with asbestos string. The importance of these precautions becomes evident when it is borne in mind that, with a closed volume on each side of the gauge, the temperature fluctuations had to be reduced to the magnitude of about 10^{-4} °C.

It was necessary also to avoid any bending of the connecting tubes when the

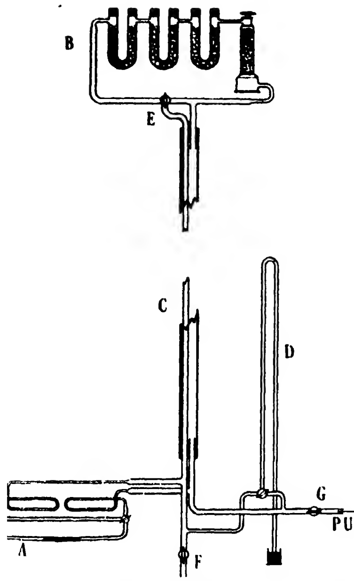

FIG. 3. *The balancing columns apparatus arranged for use as an absolute hygrometer. The two columns are shown at C, one inside the other. The outer column contains the dry air, and the inner column the air whose humidity is to be measured. The Toepler micromanometer A is connected across their bases. By suitable manipulation of the three-way cock E the tops of the columns may be connected (a) directly (for taking readings); or, (b) through the drying system B (for introducing new air samples, as explained in the text).*

gauge was being tilted, since the minute volume changes which are incurred were found to vitiate the accuracy desired. The whole apparatus was therefore mounted on the tilting table, so that it moved as a unit during adjustments of the micrometer screw.

The residual temperature effects caused persistent drifts of the gauge meniscus in one direction or the other. Since these drifts were for the most part regular, it was found possible to correct for them by taking alternate timed readings of the tilting screw when the mercury was at the upper and at the lower of two chosen positions of the capillary tube scale. By plotting these screw readings against time two roughly parallel curves were obtained. The mean of a number of readings of the distance between the curves was then taken as the number of screw divisions required to counterbalance the difference in pressure produced by a change in the mercury level from one of its two chosen positions to the other.

This procedure was followed for pressure changes ranging from 1.5 to 40 dynes per cm^2. Two different gauges with different liquids were subjected to the calibration, and the results of the test are given in Fig. 2. The straight line gives the theoretical pressure differences produced by given changes in the capillary tube assuming the compression or expansion to be purely isothermal.* The various points are the readings obtained from the gauges in the manner outlined above. The fact that the points lie upon the line within the limits of experimental error shows that the gauges were accurate within the pressure range used. This is good evidence of the reliability of this type of micromanometer.

The procedure followed in this calibration can be extended to the measurement in general of small pressure differences in confined spaces. It was found, for example, that the pressure gradients in discharge tubes could be investigated by connecting exploring tubes respectively to each side of the gauge. The gauge may also be of use in testing instruments which depend either upon the

**It might appear that the conditions of this test imply an adiabatic change, but the pressure changes were slow and were so minute that the thermal capacity of the containers rendered the operation nearly isothermal, as was found by comparison with larger and faster compressions. The insulation of the vacuum flasks was necessary, for the influence of external temperature fluctuations could not be reduced sufficiently in any ordinary thermostat.*

maintenance of constant pressure or the accurate measurement of small changes, *e.g.*, gas-thermometers and absorption hygrometers.

The Procedure with the Columns

In testing the performance of the balancing columns it was decided to return to the use of the apparatus as a hygrometer. Because of the very small difference in density between damp and dry air the precision obtained in humidity measurements is a much more rigorous test than can conveniently be devised by such methods as balancing known samples of mixed gases.

The apparatus is shown diagrammatically in Fig. 3. The two columns are shown at C, one inside the other. They had an effective height of 1213 cm., the outer consisting of an iron pipe and the inner of copper tubing, respectively 2.3 and 0.8 cm. in internal diameter; the external diameter of the inner tube was 1.0 cm. They were in contact in many places throughout their lengths, which assisted in reducing differential temperature fluctuations. The micromanometer is shown at A. B is a drying system; it consists of three large U-tubes containing pumice impregnated with sulphuric acid, and a drying tower containing phosphorus pentoxide. D is a mercury manometer, which may be connected at will to the outer column or to the inner column.

When humidity readings of the room air are to be taken, the inlet stopcock F at the base of the inner column is opened, and the three-way cock at E is turned so as to deflect an upward-flowing air stream to the left. The pump is started and the cock G is opened to an extent sufficient to cause an air stream to flow at moderate speed into the apparatus at F, up the inner column, through the drying system from left to right, down the outer column, and out to the pump. The rate of streaming is judged by the reading of the manometer D, which is connected for this purpose to the outer column. When the old air in both columns has been replaced by new samples (*i.e.*, after several minutes of streaming) the pump is stopped, F and G are closed, and E is turned through 180 degrees, thereby short-circuiting the drying system and putting the two columns into unobstructed communication at the top. The micromanometer reading is then taken, and the relative humidity is calculated from Equation (8). It may be deduced at any desired temperature merely by inserting the appropriate values of F and T*.

A series of measurements were taken in this way, and they were checked independently by a ventilated wet and dry bulb hygrometer of the Assmann type. The relative humidity given by the columns was calculated at the temperature of the dry bulb. The Assmann hygrometer was in turn checked by a chemical hygrometer.

Constancy of Readings on Repetition

The specimen readings in Table I are given to illustrate the accuracy obtained with the apparatus on repetition. Several readings of the difference

**A combination of Equations (8) and (13) indicates that at a given temperature the number of screw divisions required to balance the columns is directly proportional to the relative humidity of the air in the damp column; this shows that the apparatus can be made to read directly in percentage of relative humidity at a certain fixed temperature merely by a suitable choice of scale on the tilting screw.*

TABLE I

SAMPLE READINGS TAKEN WITH THE BALANCING COLUMNS SHOWING THE ACCURACY OF REPETITION OBTAINED

(1)	(2)	(3)	(4)
Δp dynes/cm.^{2}	Difference in density, gm./cm.^{3}	Δp dynes/cm^2.	Difference in density, gm./cm.^{3}
8 53	7.34×10^{-6}	8.85	7.44×10^{-6}
8.95	7.52	8.89	7.48
8.95	7.52	8.99	7.56
8.96	7.53	9 06	7.62
		8.91	7.50
		8.94	7.52
Mean, 8.90	Mean, 7.48×10^{-6}	Mean, 8 94	Mean, 7.52×10^{-6}

in pressure produced by two given samples of air are given in column (1), the corresponding density differences in column (2); the same two samples balanced on the following day gave the readings recorded in columns (3) and (4). The average deviation from the mean of all of the values of the difference in density given in the table is 0.055×10^{-6} gm. per cc. Such precision indicates that more elaborate temperature control is hardly necessary in ordinary measurements.

Fig. 4 gives a comparison between the values of the relative humidity obtained from the columns, and the corresponding values as measured by the Assmann instrument. A good linear agreement is shown for the humidity range covered. In this particular test the samples of damp air were all balanced against a common sample of dry air.

It will be observed that in the above test there is an average discrepancy of about 3% between the Assmann readings and those from the columns. This is due to the density of the constant sample in the reference column, which was not quite dry; it will be seen that, to obtain close agreement, it is merely necessary to move the graph parallel to itself by an amount equal to the correction. Subsequent careful repetitions and thorough preparations of the reference sample reduced this difference, but as the range covered in each of the later tests was less, the above record was

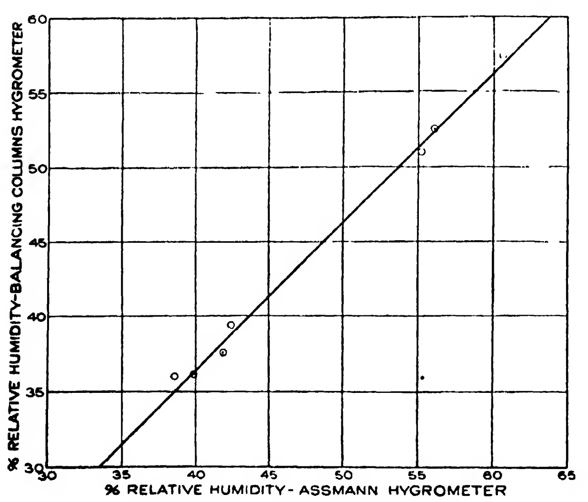

FIG. 4. *Comparison of humidity measurements of room air taken with the balancing columns and with the Assmann hygrometer.*

chosen to illustrate the linear agreement. Isolated comparisons ranged from 0% to 100% in relative humidity and, with the exception of those near saturation (for reasons already given), the same general agreement was obtained.

The departures of the points in Fig. 4 from the straight line are due to four factors: (i) experimental error in readings from the columns; (ii) experimental error in the Assmann readings; (iii) variations in the carbon dioxide content of the damp air samples; (iv) errors due to the fact that the Assmann gives the mean relative humidity of a large quantity of air, taken over a comparatively long period of time, while the columns give the humidity of only a small sample.

Of these (i) is the only one strictly associated with the balancing columns method; the other three are involved, as before, only in the means chosen to test the apparatus. Results such as those given in Table I remain the best criterion for judging the precision obtainable, and indicate that (i) is very small. It appears that the balancing columns can measure density differences to about 0.1×10^{-6} gm. per cc. even with temperature control of the simplest nature, and that the method shows promise of still greater precision if the temperature fluctuations are reduced further.

A large number of tests were made with various samples of prepared moist air, and also with mixtures of carbon dioxide, all of which provided no further information beyond confirming the usefulness and sensitivity of the method. It had been hoped to check the sensitivity closely by the use of samples of gas graded in density and measured independently, but in all such attempts the accuracy of the present method appeared greatly to surpass that of the procedure by which it was being tested.

Acknowledgment

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References

1. ROMBERG, A. and BLAU, L. W. J. Optical Soc. Am. 13: 717-724. 1926.
2. ROSECRANS, C. Z. Ind. Eng. Chem. Anal. Ed. 1: 156-158. 1929.
3. TOEPLER, A. Ann. Physik, 56: 609-643. 1895.

A PIEZO-ELECTRIC METHOD OF MEASURING THE PRESSURE VARIATIONS IN INTERNAL COMBUSTION ENGINES[1]

By H. G. I. Watson[2] and D. A. Keys[3]

Abstract

A piezo-electric pressure gauge is described in which the pressures produced by the explosions in an internal combustion engine are recorded as the displacements of the beam of a cathode-ray oscillograph. By applying a time displacement, synchronizing with the speed of the engine, to the beam so as to cause a displacement at right angles to the pressure displacements, the time-pressure characteristics of the engine explosions are obtained on a photographic film. This arrangement reduces the inertia of the gauge to a minimum and is of special value in obtaining records of a single cycle in the investigation of knocks. Specimen records taken on a Petter hot surface engine are given.

For the efficient study of the best working conditions in the modern internal combustion engine, it is necessary to determine the pressure variations in the cylinders of such engines when running under different conditions. There are many different types of indicators, but the ideal instrument should be as free from any inertia effects as possible in order that rapid variations in pressure, such as those given by knocks, may be accurately indicated. When this investigation was begun, all the methods which had been used for studying the pressure variations in internal combustion engines involved the inertia of a diaphragm. The method used by the writers involves a practically massless indicator.

The piezo-electric method of measuring pressures applies the property possessed by some crystals of becoming electrified when subjected to pressure (3, 14). The crystals of tourmaline and quartz possess this property, and when sections of these crystals are cut in the appropriate direction, it has been shown (2, 12, 13) that the charge produced on the surface of the sections is directly proportional to the total pressure applied. This piezo-electric property of tourmaline has already been used by one of the authors (6, 7) for determining the type of pressure waves formed when mixtures of water, gas and air are exploded at constant volume, and when charges of guncotton and T.N.T. are detonated under water. Karcher (5) has used the piezo-electric properties of quartz to determine the pressures in guns.

The preliminary results obtained by the method described below were presented at meetings of the Royal Society of Canada (8, 9). The method consists in having a number of quartz crystals suitably mounted in a holder which may be screwed into the engine cylinder. The pressure in the engine is communicated to the crystals without inertia, except that involved in a pressure wave passing through the crystals. The electric charge generated is directly proportional to the pressure on the crystals and is amplified by a special type

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Contribution from the Department of Physics, McGill University, Montreal, Canada, with financial assistance from the National Research Council of Canada.
[2] *Lecturer in Physics, McGill University.*
[3] *Professor of Physics, McGill University.*

of vacuum-tube amplifier, the resulting potential changes being recorded on a cathode-ray oscillograph. With this combination, the variations in pressure are transferred into similar variations in electric charge, which in turn are measured by the deflection of the cathode-ray beam of the oscillograph, the combination thus constituting a practically inertia-less pressure gauge. The cathode-ray oscillograph is so constructed that the vertical displacement of the beam is proportional to the pressure, and a horizontal motion of the beam is made proportional to the time. Such a combination was used by S. Watanabe (15) for internal combustion engines, but no attempt was made to give the pressure-time calibrated curves, the author indicating that his results showed the possibilities of the method. A more recent paper by J. Kluge and H. E. Linckh (10) gives the time-pressure curves for a rather low pressure engine, using the piezo-electric indicator and a string galvanometer for registering the variations in potential.

The apparatus used by the writers consists of the following parts: (i) the crystal detector which is screwed into the engine; (ii) the amplifier; (iii) the oscillograph; (iv) the uniform time scale apparatus; (v) the calibration apparatus.

The Crystal Detector

A diagram of the crystal detector is shown in Fig. 1, which is drawn to scale. It consists of a steel block ABC, AB being threaded to fit the spark plug opening of an ordinary gasoline engine. M is a standard spark plug with the central rod extended and bent to N as shown. The preliminary experiments were made on a Ford engine, but later a type of hot-surface Diesel engine was used, known as a Petter hot-surface oil injection engine. As this engine does not need a spark ignition, the lower part $ABVCM$ of the detector was replaced by a nipple that was screwed into the side at the top of the engine. Over the other opening is placed the thin steel diaphragm D, about $\frac{1}{8}$ in. thick, through which the elastic pressure wave is transmitted to the crystal system. The plate D acts merely as a gasket. JJ is a steel tube for holding the piezo-electric crystals, and this tube is screwed into AC. A bakelite tube SS lines the steel tube JJ. Into the lower end of SS is fitted a steel rod of the shape shown at E, and on top of this six quartz crystals F, each one inch in diameter, are placed, a piece of lead foil between each crystal. The crystals are inserted in such a way that the positive face of the first is down, *i.e.*, in contact with E. The second crystal is placed with its positive face up, the third with positive face down, and so on as indicated in the diagram. When this battery of crystals is compressed, it will be seen that three of the inside plates become negatively charged, and the other two inside lead plates and the extreme ends of the system of crystals become positively charged. The three negatively charged plates are connected by a wire which runs in a groove in the bakelite, to an insulated binding post K. The positively charged surfaces are connected together and to the metal hemisphere G, which is placed on the top. A steel cap II is screwed on the end of the tube, and a steel screw H passes through this cap and presses on G. When the crystals are all inserted, H is screwed

up very tightly so that the crystals are all pressed firmly together and the whole is pressed down against the plate D. K will now be found to be charged negatively as a result of this pressure of the screw H, but this charge is removed by connecting K with the case AB or the engine into which the apparatus

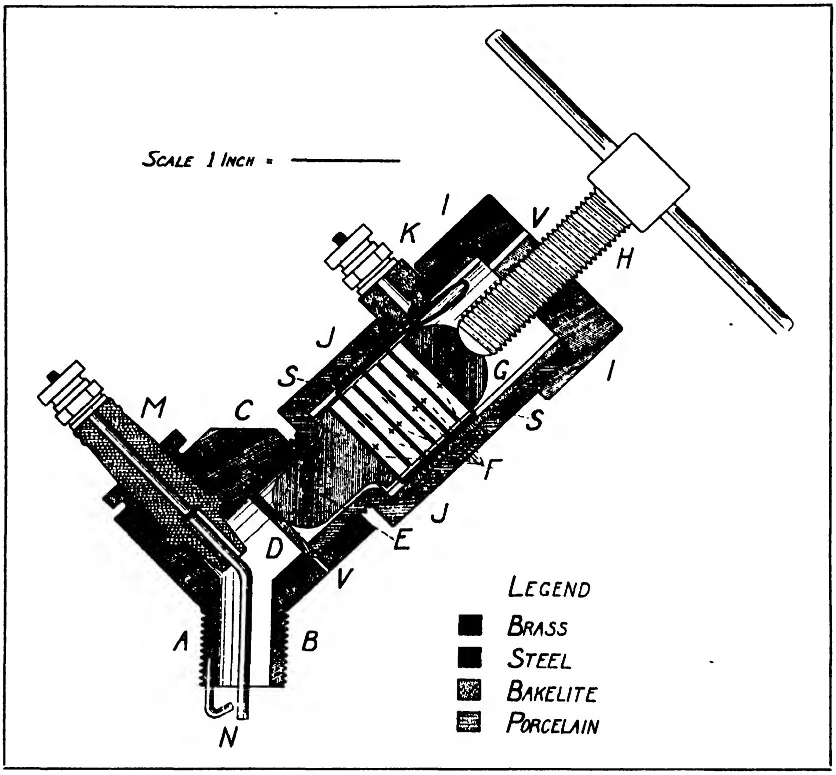

FIG. 1. *The crystal unit.*

is screwed. The pressure from the explosion in the cylinder of the engine acts on the plate D, which does not move appreciably, however, but only transmits the pressure as an elastic wave to the crystals F. This additional pressure causes K to become charged negatively with a charge of electricity which is proportional to the pressure. If the pressure on D becomes less than the normal amount, *i.e.*, if there be a rarefaction in the cylinder of the engine, then K will become positively charged with a charge which again is proportional to the rarefaction in the cylinder. Thus any pressure changes in the engine are transformed by this detector into similar variations in the potential of K, the only inertia effect in the system being the wave through the metal D and E and crystals. The natural period of vibration of the detector will be of the order of 10^{-5} sec. The binding post K is connected to a resistance-coupled amplifier, the engine being grounded. The oscillograph grounding is accomplished through the conductive networks of the amplifier circuit.

The potential to which the terminal K of the detector is raised by a given pressure will depend upon the number of crystals used. There is, however, an optimum number, n, to obtain the maximum voltage of the system for a given pressure. We have a limited length of the pressure unit for practical reasons. Suppose the length is l and the area of cross section of each crystal is A. Let q be the charge produced on each face of a crystal by the applied pressure. If K is the dielectric constant of the quartz, the capacity, c, of each crystal condenser neglecting edge effects, will be approximately $\frac{K A}{4\pi \frac{l}{n}}$, since the thickness of each crystal will be $\frac{l}{n}$. Hence $c = n \frac{K A}{4\pi l} = nx$. The capacity of the n crystals in parallel will be $nc = n^2x$ where x is a constant equal to $\frac{K A}{4\pi l}$. The total charge produced will be nq. Thus if the external capacity is C_x, we have for the voltage V, the relation $V = \frac{n q}{n^2x + C_x}$. By differentiation it follows that V is a maximum when $n^2x = C_x$, *i.e.*, when the capacity of the detector equals the external capacity. In these experiments only six plates were used, as that was the largest number that could be put into the space, the quartz available being about 3 mm. thick. About four times this number would have been better, had there been available facilities for cutting thinner quartz plates, the capacity of the detector being about 60 micromicrofarads. Such thin plates, however, would break easily, so it was considered that the thicker plates, though fewer in number, would be more practical. Also the leakage over the plates would increase with the number.

In order to keep the leakage effect as small as possible the crystal detector was shunted with a good mica condenser, the capacity of which would be varied from 0.001 to 0.006 microfarad. This reduced the potential of K, produced by the pressure, and consequently the error due to leakage of the charge was proportionally lower. These small variations in potential produced across the condenser by the charge from the crystals must then be amplified, and applied to the deflecting plates of the oscillograph.

The Amplifier

Much thoughtful work was devoted to the proper type of amplifier. for the success of the method depends on the adequate functioning of this part of the apparatus. The final form decided upon is shown in Fig. 2, and is a two-stage resistance-coupled aperiodic amplifier in which the first stage is replaced by a bridge-type arrangement. This method has the advantage that the disturbing effects of changes in battery potential, filament operating current and other small extraneous disturbing forces are minimized. It is also possible to keep the valves working on the particular part of the characteristic desired. The two-tube bridge circuit has been described by Nottingham (11), and the general arrangement of the two stage amplification by Arnold (1).

Without entering into the details of this type of amplifier here, it will be sufficient to state that the crystal detector is shown at C, and the condenser in parallel at C_x. Variations in the potential across C_x, due to pressure

changes on C, cause a change in potential of G_1, the grid of the valve T_1. This throws the "bridge" out of balance with the result that the potential of G_3 is altered causing a change in the potential across the resistance R_3. By operating the tubes on the appropriate characteristic smooth parts of their curves,

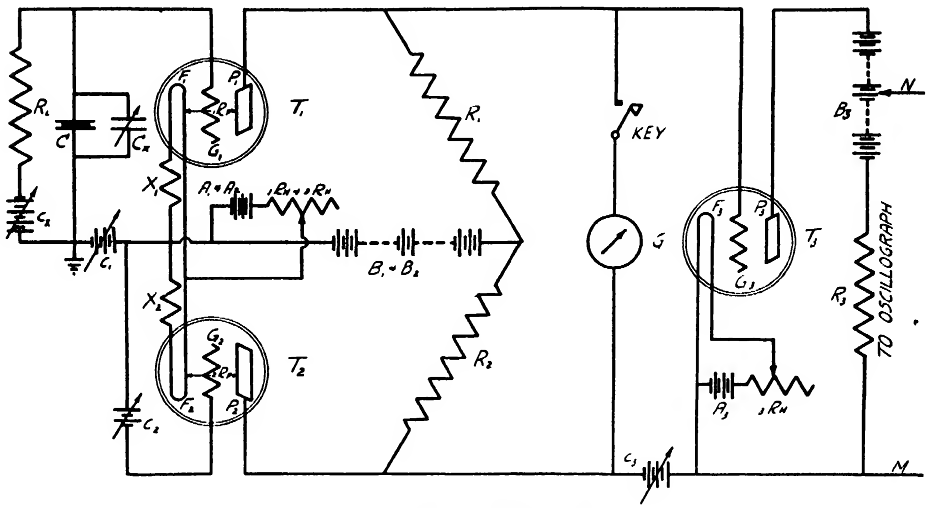

FIG. 2. *The amplifier circuit.*

it is possible to obtain a constant amplification factor for the range of changes of potential assumed by C_x due to the pressure variations in the engine. One pair of deflecting plates of the oscillograph are connected across the resistance R_3, a number of the cells of the B-battery B_3 being included in order to keep the spot, caused by the cathode-ray beam of the oscillograph, on the screen. The key and high resistance galvanometer G are included in the circuit for testing the balance of the bridge circuit, the necessary alternations in R_1, R_2, X_1, X_2 and c_2 being made so that small changes in plate battery voltages, and in the main battery rheostat, do not change the current through the galvanometer (which need not necessarily be zero). The potentials of the batteries C_1, C_2, C_3 and C_x could be varied. R_1 and R_2 were made 150,000 ohms each. R_L was 100 megohms, X_1 and X_2 being small adjustable resistances for compensating any small variations in filament battery voltages. R_3 was 100,000 ohms. The battery voltage B_1B_2 was 180 volts, and B_3 also 180 volts of which about 100 were included in the oscillograph circuit. The characteristics of each valve were determined and they were operated on the linear portion of the plate current-grid voltage curve. Since the apparatus was calibrated *in situ*, the actual amplification of the system need not be known.

The Oscillograph

A low voltage cathode-ray oscillograph of the J. B. Johnson type, such as that supplied by the Western Electric Company, was used (4). This instrument is provided with two pairs of deflection plates very nearly at right angles. The

one pair of plates is connected to the amplification terminals NM, Fig. 2. This pair was so arranged that the deflections due to variations in potential across NM were in a vertical plane. The vertical deflection of the spot on the screen was thus proportional to the potential across NM, and this in turn was directly proportional to the potential across the capacity C_x, which varies, as already stated, as the pressure in the cylinder of the engine. Hence the vertical displacements of the cathode-ray beam are proportional to the pressures in the engine.

The second pair of plates in the oscillograph are connected to the timing device and these plates cause the spot to move in a horizontal direction with uniform velocity. The timing could be altered so that the time required for the beam to swing from one end of its path to the other agreed with a single cycle of the engine, and thus a time-pressure pattern, which would appear stationary to the eye, would be traced on the screen. By having the oscillograph in a dark room, it was found that the amount of light from a single transit of the beam on the willemite screen was sufficient to leave a record on a photographic film, when the film was placed with its sensitive side directly against the glass screen of the oscillograph. As a rule the pattern repeated so well that several transits could be photographed, giving a consistent uniform picture on the film. When artificial knocks were produced the single excessive pressure, due to the premature explosion, was plainly visible on the photographic record. For reproduction purposes, it is necessary to give multiple transit pictures, which are more diffuse than the single transit records.

The Uniform Timing Circuit

The timing circuit which was used is shown in the diagram, Fig. 3. The terminals F and G are connected to the time deflection plates of the oscillograph, G being the common terminal of the plate system of the Johnson oscillograph. D is a voltage regulating tube of the "Radiotron" UX 874 type. B is a battery, the voltage of which may be varied from 90-135 volts, to regulate the mean voltage across the condenser C, which is also the potential across FG, in order that the cathode-ray beam will describe a path symmetrically, between the two deflecting plates of the oscillograph, of the proper length. The capacity of the condenser C could be varied from 0.001 to 1000 microfarads. The terminals YY were short circuited during an exposure, but a contact on the flywheel of the engine could be inserted between YY in order to give on the oscillograph the position of the piston with respect to the time scale. A 300-volt battery connected in series with a variable high resistance R (0.02 to 20 megohms) was connected across the condenser C. We thus have

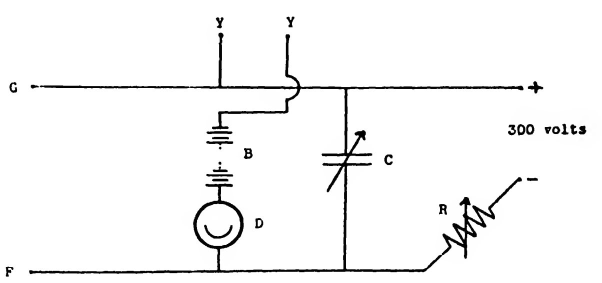

FIG. 3. *The time scale circuit.*

the equation for the voltage V across the condenser C at any time t from the moment the battery is connected given by $V=E\,(1-\epsilon^{-\frac{t}{RC}})$, where E is the total potential of the battery (300 volts), C the capacity of the condenser and R the resistance. By altering the values of R and C, the time constant of the circuit may be arranged so that the periodicity of the breakdown of the tube D will coincide with the period of the cycle of the engine. By the choice of this resistance and capacity with the voltage E being 300 volts, only the first part of the exponential growth curve is used and this may be made to differ from a linear relation by a negligible amount. In the apparatus used it was practically linear within the limits of the experimental error in measurement.

With this timing device stationary figures of the time-pressure curve were obtained on the oscillograph when the engine was running at a uniform speed. It was found incidentally that the regularity of the neon voltage regulating tube was very much improved when the tube was illuminated with the light from a 40-watt lamp. This was apparently due to a small photo-electric effect which made the tube break down with precision when a definite potential was reached. Such a lamp was placed beside the Radiotron tube, and the whole enclosed in black cloth to keep the room dark during the taking of a photographic record.

The Calibration of the Crystal Detector

The crystal detector was removed from the engine and screwed into a special form of hydrostatic calibration apparatus shown in Fig. 4, the whole system remaining otherwise in the same condition as used in obtaining the oscillographic record of the time-pressure variations in the engine. The pressures were applied to the detector by screwing up the plunger until the oil pressure reached a given value as indicated by the gauge. The charge was then removed from the oscillograph plates and the pressure suddenly released by opening the valve. Under these conditions the charge produced on the detector will be equal, but of opposite sign, to that produced by applying the same pressure. Since the pressure can be released more rapidly than applied, this method was used in calibrating the apparatus, as the time for any leakage was reduced to a minimum. The deflection of the spot on the oscillograph was then measured for a given pressure. In this way there was obtained a pressure

FIG. 4. *Pressure calibration apparatus.*

calibration curve which was found to be practically linear. Two examples of such curves are given in Fig. 5. When the batteries become run down, the curve is no longer linear. The calibration curve taken when the above records were made is shown in Fig. 5, *B*, the calibration curve *A* representing the results when the battery potentials are well up. The capacity in the circuit in the two cases was different, the capacity used in the case of curve *B* was 0.004 microfarads. This method of calibration avoided the necessity of determining the amplification factors, capacity of the system and other constants, since these quantities all remained constant.

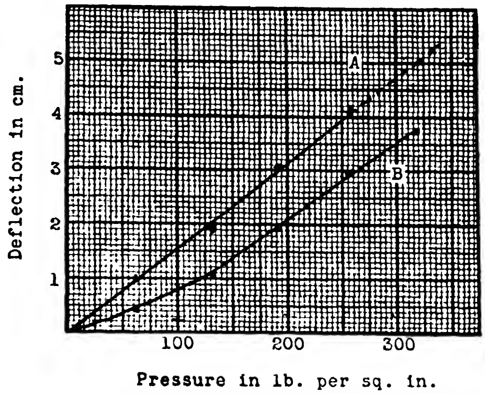

FIG. 5. *Pressure displacement calibration curves.*

The gauge was later calibrated against known pressures applied on an apparatus loaned for the purpose by Dean Ernest Brown of the Engineering Faculty. It was found that the authors' gauge did not read correctly, but a good correction curve was obtained. It thus became possible to transfer the oscillograph pressure displacements into actual pressures measured in pounds per square inch, as shown in Fig. 5.

Results

A number of photographs were taken of the time-pressure curves with the apparatus described above. The Petter hot-surface engine, of the Mechanical Engineering Laboratory at McGill University, was used, and records were obtained for different loads and also for various values of the capacity in parallel with the crystal detector. Records of knocks artificially obtained in the engine were also secured.

To illustrate the type of result which has been achieved, prints from two typical records are shown in Figs. 6 and 7 taken under similar working conditions of the engine, but with different values of the capacity in parallel with the detector. The net load on the brake balance in each case was 20 lb. It will be seen that both curves have the same general shape but the one shown in Fig. 7 has a higher maximum displacement than the other. This is due to the smaller capacity used in taking the record shown in Fig. 7. The pressure displacements in the two cases will be inversely proportional to the capacities used. The capacity used in the first case was 0.004 microfarad, and in the second 0.003 microfarad, their ratio being 1.33. Thus 1.4 is approximately the ratio of the maximum displacements.

These records are time-pressure curves but it is usual to indicate the volume-pressure relations. The time scale has thus to be converted into a volume scale. This may be done by using the contact on the flywheel of the engine

in the timing device shown at *YY* in Fig. 3. The position of this contact on the wheel relative to the position of the piston in the cylinder is known and thus a definite point on the time scale is related to the position of the piston. From a knowledge of this fact, it is possible to transfer the time scale to a volume scale. This has been done in the case of the curve shown in Fig. 6, supposing the crank to be very much smaller than the connecting rod, which is approximately so in this engine. For accurate computation a method such as that suggested by Kluge and Linckh (10) must be followed. The results are shown in Fig. 8. In Figs. 6 and 7 the pressure displacements will have different scales, owing to the different capacities used in taking the records as already explained. By comparing the maximum displacements with the calibration curves the maximum pressure in the case of Fig. 6 was found to be 314 lb. per sq. in., whereas in the case of Fig. 7 it was 307 lb. per sq. in., a result in good agreement. These curves indicate that the method duplicates observations satisfactorily. For comparison, a print from a record taken on this engine seven years ago with the Cambridge Mechanical Indicator is shown in Fig. 9. In shape at least the two methods give similar results.

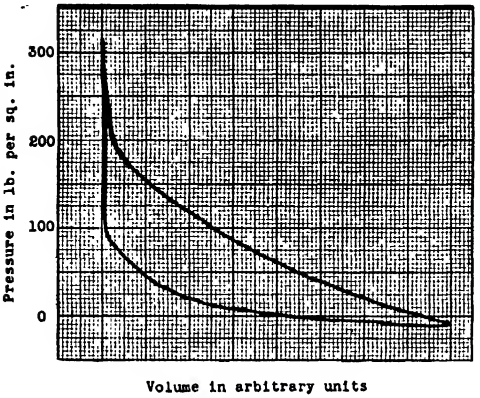

FIG. 8. *Pressure-volume curve by piezo-electric gauge.*

To compare the authors' results with the maximum pressures obtained by using the Bureau of Standards type of balanced pressure diaphragm indicator, Professor R. H. Patten, of the Mechanical Engineering Department, very kindly took the readings shown in Table I, with the engine used by the writers.

TABLE I

RESULTS OBTAINED WITH THE WRITERS' ENGINE USING THE BUREAU OF STANDARDS TYPE OF BALANCED PRESSURE DIAPHRAGM INDICATOR

Load, lb.		2	5	10	15	18	21
Readings, lb. per sq. in.	Min.	180	210	220	290	300	315
	Max.	260	250	260	300	310	320

The authors used a load of 20 lb. in obtaining the curves shown in Fig. 6 and 7; the mean result is about 311 lb., a value in good agreement with the above.

Records of knocks artificially produced by pre-ignition were obtained. The record is perfectly clear, but the single transit does not affect the film sufficiently strongly for reproduction purposes. As an example, for one record the maximum pressure was 321 lb. per sq. in., while the engine was running, the pressure rising at the knock to 562 lb. per sq. in. Such a knock is a very

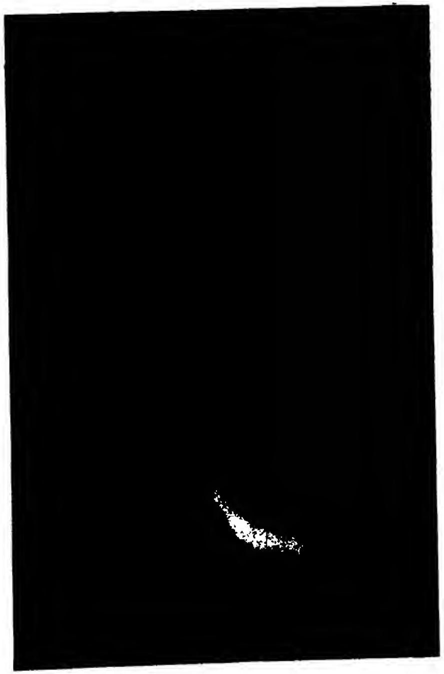

FIG. 6. *Time-pressure curve using a shunting capacity of 0.004 microfarad.*

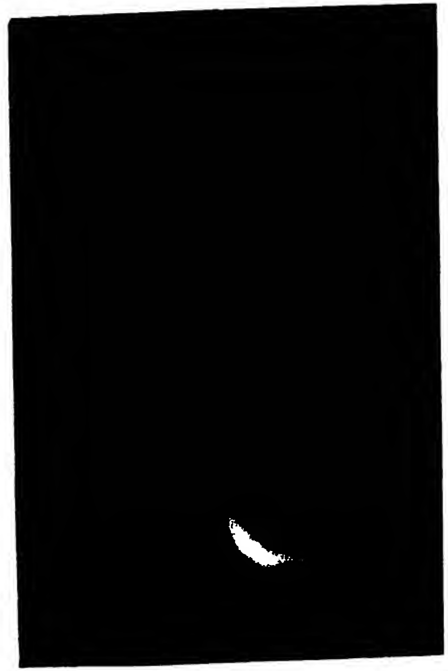

FIG. 7. *Time-pressure curve using a shunting capacity of 0.003 microfarad*

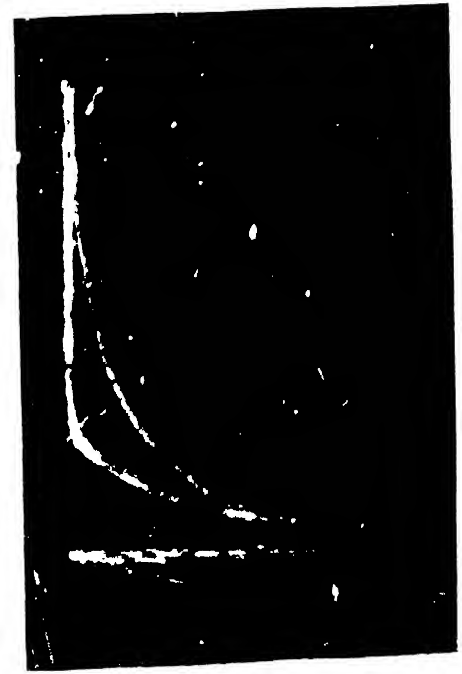

FIG. 9. *Cambridge mechanical indicator diagram of same engine.*

sudden affair, and it is under these conditions that the method using the cathode-ray oscillograph has an advantage over any mechanical indicator, or a device in which a galvanometer is used.

The results obtained in this investigation show that the piezo-electric pressure gauge in conjunction with the cathode-ray oscillograph may be successfully used as an inertia-less indicator for internal combustion engines. The new Von Ardenne cathode-ray oscillograph, owing to the greater intensity of its beam, would give single transit records that could readily be photographed.

Acknowledgment

The writers wish to express their thanks to the Department of Mechanical Engineering, McGill University, for permission to use the engine on which these tests were made.

References

1. ARNOLD, H. D. U. S. Patent 1,129,943. 1915.
2. CROSSLEY, A. Proc. Inst. Radio Eng. 15: 9-36. 1927.
3. CURIE, PIERRE. Oeuvres. Paris. Gauthier-Villars, pp. 15-55. 1908.
4. JOHNSON, J. B. J. Optical Soc. Am. 6: 701-712. 1922.
5. KARCHER, J. C. Scientific Papers of the Bureau of Standards. No. 445. 1922.
6. KEYS, D. A. J. Franklin Inst. 196: 577-592. 1923.
7. KEYS, D. A. Phil. Mag. 42: 473-488. 1921.
8. KEYS, D. A. and WATSON, H. G. I. Proc. Roy. Soc. Can. 21: lxxvii. No. 4. 1927.
9. KEYS, D. A. and WATSON, H. G. I. 1927. Presented at Roy. Soc. Can. May Meeting. 1931.
10. KLUGE, J. and LINCKH, H. E. Z. Ver. deut. Ing. 74: 887-889. 1930.
11. NOTTINGHAM, W. B. J. Franklin Inst. 209: 287-348. 1930.
12. POYNTING, J. H. and THOMSON, J. J. A Textbook of physics, Vol. IV. Electricity and magnetism, pp. 148-163. Chas. Griffin and Co. London. 1914.
13. VIGOUREUX, P. Quartz resonators and oscillators, pp. 1-28. Lond. H. M. Stationery Office. 1931.
14. VOIGT, W. Lehrbuch der Kristallphysik, pp. 801-827. Teubner. Leipzig. 1910.
15. WATANABE, S. Scientific Papers of the Inst. of Phys. Chem. Research, 12: 82-112. 1929.

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VOLUME 6 APRIL, 1932 NUMBER 4

THE RELATIVE MILLING AND BAKING QUALITY OF WESTERN CANADIAN SPRING WHEAT VARIETIES[1]

BY J. G. MALLOCH[2], W. F. GEDDES[3] AND R. K. LARMOUR[4]

Abstract

To maintain the quality of Canada's export wheat it is essential that only high quality varieties should be grown. To supply information on which a choice of varieties may be based, a co-operative study was made of the milling and baking quality of 25 varieties of spring wheat now grown in western Canada. Samples were grown in adjacent plots by the Dominion Experimental Farms and Universities in Manitoba, Saskatchewan and Alberta in 1928, 1929 and 1930. Only samples which were sound enough to be placed in the statutory grades by official inspectors were used. Part of each sample was milled and baked in each of the three co-operating laboratories. Four baking formulas were used. The varieties were classified on the bases of loaf volume, texture, crumb color, general appearance of loaf, absorption, and yield of straight flour. These classifications were combined to give classifications for baking quality and milling quality and finally for suitability for export and domestic milling. The last classification is given in Table XXVII and is, briefly, as follows:

1. Varieties which are entirely satisfactory: Reward, Ceres, Marquis, Pioneer, Red Fife, Renfrew, Red Bobs 222, Supreme.
2. Varieties which are fairly satisfactory: Early Red Fife, Ruby, Early Triumph.
3. Varieties which are unsatisfactory: (a) White wheats: Quality, Axminster, Hard Federation; (b) Varieties differing from Marquis in milling characteristics: Garnet, Kota; (c) Varieties inferior to Marquis in baking characteristics: Garnet, Parker's Selection, Brownhead, Huron, Kitchener, Preston, Marquillo.
4. Varieties which are very unsatisfactory: Early Prolific, Dicklow, Vermilion.

Introduction

The high reputation for quality of Canadian wheat was established when the major part of the production in western Canada was limited to one variety, Red Fife, grown in a comparatively small area. The extension of

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wheat growing westward and northward was favored by the introduction of an earlier-maturing variety, Marquis, which fully sustained the reputation established by Red Fife.

In recent years the enormous losses occasioned by plant diseases, insect pests, and drought, together with the spread of the wheat-growing area northward into regions where early frosts are a yearly menace, led to the development and introduction of new varieties in an effort to overcome these difficulties.

Many of the newer varieties differ from Marquis in shape, size and color of kernel and as a result there was a decrease in the uniformity of appearance of our export wheat. Uniformity is an important factor in the eyes of the buyers and varietal mixing has been the basis of complaints with regard to the quality of export shipments. This has naturally led to the question as to whether the new introductions are equal to Marquis in milling and baking quality. The present investigation was undertaken to obtain an answer to this question and to give a basis for the elimination of inferior varieties.

This paper deals with the quality of an extended series of varieties grown in 1928, 1929 and 1930.

Materials and Methods

Collection of Samples

In order that valid comparisons may be made between varieties it is essential that the samples compared should be grown under identical environmental conditions. It is a well-known fact that weather and soil conditions exert a profound influence on the chemical composition, and milling and baking quality of wheat. The variation in quality in samples of a single variety grown at different places is so great that varieties to be compared must be grown in adjacent plots. Furthermore, since the varieties in a series of samples grown in different places will not always fall in the same order with respect to quality it is unsafe to draw conclusions from the results of a single series. Finally a sound sample of one variety should not be compared with a damaged sample of another variety.

For this study samples were obtained from adjacent plots of the varieties under test grown at the Universities and Experimental Farms in the three prairie provinces in the years 1928 to 1930 inclusive. These samples were graded by inspectors of the Western Grain Inspection Division and only samples which graded into the statutory grades were used in our comparisons.

Co-operating Stations

University of Alberta, University of Manitoba, University of Saskatchewan, and the Dominion Experimental Farms at Beaverlodge, Brandon, Fort Vermilion, Indian Head, Lacombe, Lethbridge, Morden, Rosthern and Scott.

Varieties Tested

Axminster, Brownhead, Ceres, Dicklow, Early Prolific, Early Red Fife, Early Triumph, Garnet, Hard Federation, Huron, Kitchener, Kota, Marquillo, Marquis, Parker's Selection, Pioneer, Preston, Quality, Red Bobs 222, Red Fife, Renfrew, Reward, Ruby, Supreme, Vermilion.

Criteria of Quality

A wheat of good quality is one which will produce a high yield of flour of good baking quality. This definition is far from precise. Although the possible yield of flour from any wheat is the main factor in milling quality, the miller also considers the tempering properties, the capacity to blend well with other wheats and the power required in milling. Baking quality is a relative term and there is no absolute measure of this important characteristic. One has to consider the suitability of a flour for large machine bakeries, small hand bakeries, home baking, straight dough and sponge dough systems, long and short fermentations, and the particular type of loaf desired in any given locality. However the quality of a sound sample of Marquis wheat grown under favorable climatic and soil conditions is universally accepted as the standard of quality for Canadian wheat. This gives a standard of comparison which is independent of local conceptions of quality.

The milling quality of the varieties was determined by the yield of straight flour obtained in a milling test. The other factors in milling quality outlined above were not determined quantitatively but were the subject of general observation by the millers making the tests.

The baking quality was judged by the loaf volume, absorption, texture and color of crumb and the general appearance of the loaf, which includes the shape, and the color of the crust. Information on these points was obtained using four different formulas in the baking procedure. While it is true that the handling qualities and the stability and elasticity of the dough are important factors in baking quality it is exceedingly difficult to measure them or to express them numerically. The use of four baking formulas giving, in effect, a wide range of baking conditions, makes it extremely unlikely that inherent weakness in any of the varieties studied remained undiscovered.

In addition to using these primary criteria of quality, the varieties were compared on the basis of weight per measured bushel, which is universally used as an index of milling yield, and on the basis of the protein content of the wheat, which is regarded, on the American Continent, as a good general index of baking quality.

Methods

The samples received from the co-operating stations were cleaned at the collecting laboratory and then divided into three sub-samples which were distributed to the three co-operating laboratories. Our results are therefore based on the average of three independent tests.

The details of our methods of analysis and milling and baking tests have been given fully in several published papers (1, 3, 4) and it is unnecessary to repeat them here. It seems desirable, however, to indicate the nature of the four formulas used in the baking tests.

1. *Simple:* flour, yeast, salt, sugar and water. This formula gives information on the quality of the flour unaffected by the addition of "improvers". If used as the sole criterion of quality, however, it may lead to entirely erroneous comparisons. Flours with a high protein content which are deficient in gas production seldom show their maximum capabilities with this formula.

2. *Bromate:* simple formula plus potassium bromate. The action of the potassium bromate is as yet obscure but it is known that it directly or indirectly affects the protein. It is the basis of some improvers in wide commercial use. This formula appears to be particularly adapted for the estimation of quality of high protein Canadian wheat.

3a. *Malt:* simple formula plus non-diastatic malt. This formula was used with the 1928 samples.

3b. *Malt-phosphate:* simple formula plus small percentages of diastatic malt and ammonium phosphate. This formula was used with the 1929 and 1930 samples.

Both of these formulas are designed to insure an adequate supply of gas for raising the dough. Flours with a high percentage of protein of good quality but which are deficient in gas production will show to advantage when baked by these formulas.

4. *Blend-bromate:* flour blended with 50% soft wheat flour and then baked by the bromate formula. Baking tests using this formula give information on the ability of the test flour to "carry" a weaker flour. This is important since the bulk of our wheat is sold for export and is blended either before or after milling.

Analysis of Results

Introductory Discussion

The restriction of the samples to grade Four Northern or better seriously cut down the number of available samples in 1928 owing to the number of frosted samples and to a lesser extent in the other years. Also, for various reasons, the entire series of samples was not grown at all stations in all three years. It was therefore impossible to compare all the varieties by a single statistical analysis. Hence, it was necessary to split the series into overlapping groups in which valid comparisons could be obtained, analyzing the results for each group separately. These were then combined by reference to the results for varieties which were common to a number of these groups. By using this method varieties were compared directly only when they had been grown under identical conditions. Once the relative quality of the varieties for each group has been determined in this manner the combination into one classification containing all the varieties studied is valid, provided that the order of the varieties and not the numerical results is considered.

The results in each group were analyzed by the application of Fisher's analysis of variance (2).

In the analysis of variance the total variability of an experiment is expressed as a sum of squares of the deviations of each individual determination from the general mean. This sum of squares is then divided up into component parts and each of these sums of squares may be used to estimate the variability of the experiment by calculating for it a variance (which is the sum of squares divided by the corresponding number of degrees of freedom). The differences between these estimates of the variance bring out the important factors in the experiment and their significance can be measured by means of the Z test.

For any two estimates of the variance the Z value is one-half the difference between their natural logarithms. If the Z value obtained equals or exceeds the corresponding Z value at the 5% (or 1%) point, it may be said that the observed difference between the estimates of variance would occur, by chance, in only 5% (or 1%) of the cases in a large number of trials.

This method of analysis made it possible to study the differences between varieties, entirely separate from the variation in one variety grown at different places or baked by different formulas. Further, it was possible to get an accurate measure of the effect of different growing conditions, occasioned by the samples being grown in different places and different years, on the constancy of the order in which the varieties fall with respect to any particular character. The method has also the advantage that it gives an accurate estimate of the error of the experiment and hence of the significance of the differences found. A difference of more than three times the standard error is considered to be significant. In making the classification for any given character, Marquis was used as the standard. Varieties falling above (or below) this standard were regarded as superior (or inferior) in that particular character. Varieties falling below Marquis may be adapted to special uses, but from the standpoint of the quality desired in our export wheat they must definitely be regarded as inferior.

Analysis of Baking Data

In analyzing the results of the baking tests each of the five baking characteristics was studied separately. When the restrictions in regard to year and place of growth and soundness of samples, discussed in the foregoing section, were imposed upon the comparison of varieties, these fell into thirteen groups, all containing the varieties Early Triumph, Huron, Marquis, Red Bobs 222 and Reward. The data for each group were subjected to an analysis of variance, an example of which is given in Table I. It will be seen that no attempt was made to study the variation between years or between places.

TABLE I
ANALYSIS OF VARIANCE
LOAF VOLUME

-	Sum of squares loaf volume	Degrees of freedom	Variance	$\frac{1}{2}$ Log_e	Z
Series (S)	4606.87	13	354.37	2.9352	1.8382
Varieties (V)	2332.77	4	583.19	3.1842	2.0872
Formulas (F)	10799.04	3	3599.68	4.0943	2.9973
V × F	815.17	12	67.93	2.1092	1.0122
F × S	2381.16	39	61.05	2.0559	0.9589
V × S	1171.63	52	22.53	1.5574	0.4604
Error	1399.63	156	8.97	1.0970	—

Varieties:– Reward, Marquis, Red Bobs 222, Early Triumph, Huron.
Series:– Lacombe 1930, Edmonton 1929 and 1930, Beaverlodge 1930, Saskatoon 1929 and 1930, Scott 1929 and 1930, Swift Current 1930, Indian Head 1930, Rosthern 1929 and 1930, Winnipeg 1929, Brandon 1929.

Each series was regarded as having been grown under different environmental conditions even though in many cases series were grown in the same place but in different years.

In studying the relative qualities of the varieties the important portions of the variance are:

1. Variance between varieties. By comparing this with the variance due to error the significance of the differences between varieties can be estimated.
2. Interaction of varieties and series (V × S). This gives a measure of the constancy of the order of the varieties when grown under different environments.
3. Interaction of varieties and formulas (V × F). This gives a measure of the constancy of the order of the varieties when baked by different formulas.
4. Variance due to error. This represents the portion of the total variance which cannot be satisfactorily accounted for by known causes of variation. It is used in estimating the significance of the other portions of the variance and also in estimating the significance of the differences between the varieties by means of the standard error which is calculated from it.

Analyses of variance for loaf volume similar to that shown in Table I were made for each of the 13 groups previously mentioned. The results are summarized in Table II in terms of the ratio of the Z value obtained to the Z value at the corresponding 5% point, ratios of 1.0 or greater being significant.

When the varieties in each group are arranged in relative order a compilation such as that in Table IV for loaf volume is obtained. It will be observed that the absolute values for any given variety vary from group to group, owing to the fact that these groups represent different combinations of varieties, stations, and years. The minimum significant differences were used as units for placing the varieties in classes with respect to Marquis as a standard. This placing is given for loaf volume in Table V. The procedure may be more easily followed in a specific example. In Table IV (Group 1) the varieties fall in the order Reward, Marquis, Red Bobs 222, Early Triumph and Huron. The significant difference between varieties is 12 cc. The average volumes of these varieties are 666, 628, 616, 600 and 580 respectively. It will be seen that the difference between Reward and Marquis is between 3 and 4 times the significant difference and Reward was accordingly placed in class +3 in Table V. Similarly, Red Bobs 222 which is just significantly different from Marquis is placed in class −1, Early Triumph in class −2 and Huron in class −4. All the other groups in the table were treated in a similar manner and the same procedure was followed in subsequent tabulations of other characters. These classifications together with the information in the tables of averages were used as the bases for single classifications for each characteristic.

Loaf Volume

Turning now to a more detailed consideration of the data for loaf volume it will be noted from Table II that all the components of variance are significant. The differences between varieties are much greater than could have occurred merely by chance. The interaction between variety and formula is highly

significant. This means that the formula used has a pronounced influence on the order in which the varieties fall with respect to loaf volume. While the averages of all formulas have been used in the classifications, important differences in the results of the four formulas will be noted in the description of the individual varieties. The interactions between variety and series are also statistically significant. That is to say, the order of the varieties with respect to loaf volume will vary when the varieties are grown at different places or in different years. It should be pointed out, however, that the level of significance is, in this case, comparatively low and that in practice the variation

TABLE II

SIGNIFICANCE OF COMPONENTS OF VARIANCE
LOAF VOLUME

Group	Number of series	Ratio of Z obtained to Z at 5% point					
		Series (S)	Varieties (V)	Formulas (F)	Interactions		
					V × F	F × S	V × S
1	14	6.4	4.7	6.1	3.4	4.9	2.6
2	11	4.8	5.1	6.3	3.9	4.5	2.3
3	11	6.2	5.2	6.2	3.5	5.3	2.7
4	8	3.2	5.5	5.5	3.8	2.9	2.3
5	12	5.8	4.7	6.0	3.8	5.1	2.5
6	10	5.4	4.5	5.9	4.0	4.3	2.5
7	13	5.7	4.4	6.0	4.1	4.0	2.4
8	10	4.2	4.7	6.3	3.7	4.3	1.5
9	11	5.9	4.7	6.2	3.5	5.4	3.0
10	7	4.2	4.2	5.6	2.5	4.4	1.3
11	5	1.7	4.6	5.0	3.3	1.3	0.0
12	6	4.8	3.8	5.7	3.2	3.7	1.9
13	6	4.6	4.0	5.9	3.9	3.5	2.6

in order from series to series is not sufficient to invalidate our general conclusions. In Table IV (Group 1) it will be seen that the average loaf volumes fall in the order Reward, Marquis, Red Bobs 222, Early Triumph and Huron. The position of each of these varieties for each station and each year entering into the analysis was determined and the frequency with which each variety fell in each of the five positions is shown in Table III.

TABLE III

FREQUENCY OF POSITION IN 14 SERIES OF COMPARISONS OF LOAF VOLUME (AVERAGE) FOR REWARD, MARQUIS, RED BOBS 222, EARLY TRIUMPH AND HURON

	1st	2nd	3rd	4th	5th
Reward	12	2	0	0	0
Marquis	2	7	2	3	0
Red Bobs 222	0	3	10	1	0
Early Triumph	0	1	2	9	2
Huron	0	1	0	1	12

TABLE IV

AVERAGE LOAF VOLUME OF EACH VARIETY IN EACH GROUP

Loaf volume, cc.	Group 1	2	3	4	5	6	7	8	9	10	11	12	13
Minimum significant difference (cc.)	12	14	16	17	14	15	14	13	14	15	25	17	17
680–689		Reward									Reward		
670–679			Kota	Reward			Reward	Reward					
660–669	Reward					Reward				Reward		Reward	Reward
650–659			Reward		Reward				Reward Ceres		Marquis		
640–649				Marquis				Marquis					
630–639		Marquis Red Fife				Marquis	Marquis						
620–629	Marquis	Red Bobs 222 Renfrew	Axminster Marquis	Red Bobs 222				Red Bobs 222 Early R. Fife	Marquis	Marquis	Red Bobs 222	Marquis	Marquis
610–619	Red Bobs 222		Marquillo Parker's Sel.		Marquis	Red Bobs 222	Red Bobs 222 Supreme			Red Bobs 222	E. Triumph	Red Bobs 222 E. Triumph	E. Triumph
600–609	E. Triumph	E. Triumph	Red Bobs 222 Preston	E. Triumph	Red Bobs 222 E. Triumph	E. Triumph	E. Triumph	E. Triumph	Red Bobs 222 Quality E. Triumph	Ruby E. Triumph			Red Bobs 222
590–599		Kitchener Huron	E. Triumph	Huron							Huron		Brownhead
580–589	Huron		Huron			Huron Hard Fed'tion	Huron	Huron				Huron	Huron Early Prolific
570–579					Garnet				Huron				
560–569					Huron					Huron			
550–559													
540–549													
530–539													
520–529													
510–519				Dicklow									
500–509										Vermilion			

TABLE V

Classification on basis of minimum significant difference from Marquis

Loaf volume

Class	Group 1	2	3	4	5	6	7	8	9	10	11	12	13
Minimum significant difference (cc.)	12	14	16	17	14	15	14	13	14	15	25	17	17
+3	Reward	Reward	Kota		Reward								
+2			Reward			Reward	Reward	Reward	Reward Ceres	Reward			Reward
+1				Reward							Reward	Reward Pioneer	
0	Marquis	Marquis Red Fife Red Bobs 222	Axminster Marquis Marquillo Parker's Sel. Red Bobs 222	Marquis	Marquis Red Bobs 222	Marquis	Marquis Red Bobs 222	Marquis	Marquis	Marquis Red Bobs 222	Marquis	Marquis Red Bobs 222 E. Triumph	Marquis E. Triumph Red Bobs 222
−1	Red Bobs 222	Renfrew	Preston E. Triumph	Red Bobs 222	E. Triumph	Red Bobs 222 E. Triumph	Supreme E. Triumph	Early R. Fife Red Bobs 222	Red Bobs 222 Quality E. Triumph	E. Triumph Ruby	Red Bobs 222 E. Triumph		
−2	E. Triumph	E. Triumph Kitchener	Huron	E. Triumph	Garnet	Huron		E. Triumph			Huron	Huron	Brownhead Huron Early Prolific
−3		Huron		Huron	Huron	Hard Fed'tion	Huron		Huron	Huron			
−4	Huron							Huron					
−5												Vermilion	
−6													
−7				Dicklow									

TABLE VI

CLASSIFICATION OF VARIETIES ON BASIS OF LOAF VOLUME

1. *Superior to Marquis*
   Kota
   Reward
   Ceres
   Pioneer

2. *Equal to Marquis*
   Axminster
   Marquis
   Marquillo
   Red Fife, Parker's Selection

3. *Slightly inferior to Marquis*
   Red Bobs 222
   Supreme, Early Red Fife
   Renfrew
   Preston, Quality
   Early Triumph, Ruby

4. *Decidedly inferior to Marquis*
   Kitchener, Brownhead
   Garnet
   Huron, Early Prolific
   Hard Federation

5. *Very much inferior to Marquis*
   Vermilion
   Dicklow

It will be seen that there is some variation in position but there is little doubt that the general conclusion that the varieties fall in the order previously named is correct. A similar argument can be applied to the other varieties.

The information contained in Table IV was brought to a common basis by the method previously outlined and the results are given in Table V.

From Table V the single classification in Table VI was compiled. The order of the varieties in each class is that arrived at from the data, but the differences between the varieties within any one class probably have no practical significance. However, in the further combinations of the classifications it is useful to know whether the variety fell near the top or the bottom of any class.

Texture

Table VII shows significant differences in the texture of varieties and significant interaction between variety and formula. The interaction between variety and series is less significant than in the case of loaf

TABLE VII

SIGNIFICANCE OF COMPONENTS OF VARIANCE

TEXTURE

Group	Number of series	Ratio of Z obtained to Z at 5% point					
		Series (S)	Varieties (V)	Formulas (F)	Interactions		
					V × F	F × S	V × S
1	14	4.2	2.7	5.7	2.0	4.7	0.9
2	11	4.3	2.9	5.9	1.8	4.0	0.7
3	11	5.4	2.6	6.1	2.8	6.3	1.8
4	8	3.9	3.4	5.6	1.5	3.7	0.6
5	12	3.5	2.9	5.5	2.1	4.6	1.7
6	10	4.4	2.4	5.5	1.8	5.2	0.5
7	13	4.1	3.5	5.8	3.0	5.5	2.5
8	10	3.7	2.4	5.7	2.5	3.9	1.0
9	11	3.4	2.0	5.5	2.1	4.8	0.8
10	7	3.4	2.3	5.4	1.9	3.6	1.5
11	5	2.6	3.6	3.8	1.5	2.0	0.0
12	6	3.3	1.6	4.5	1.4	3.7	0.5
13	6	3.3	3.2	4.6	1.9	3.6	0.0

TABLE VIII

AVERAGE TEXTURE SCORE OF EACH VARIETY IN EACH GROUP

Texture score	Group 1	2	3	4	5	6	7	8	9	10	11	12	13
	Minimum significant difference												
	0.28	0.30	0.28	0.35	0.28	0.28	0.25	0.29	0.30	0.32	0.50	0.37	0.37
7.5													
7.4		Renfrew					Supreme			Marquis			
7.3			Reward		Reward				Reward Marquis		Marquis	Marquis	Reward
7.2	Reward	Reward			Marquis	Hard Fed'tion Reward	Reward	Marquis Reward			Reward	Reward	Marquis
7.1	Marquis	Kitchener Marquis		Reward			Marquis		Ceres	Reward Ruby		Pioneer	
7.0			Marquis Axminster	Marquis		Marquis			Red Bobs 222		E. Triumph Red Bobs 222		E. Triumph Red Bobs 222 Brownhead
6.9			Kota E. Triumph Red Bobs 222		Red Bobs 222 E. Triumph			Early R. Fife	Quality E. Triumph	Red Bobs 222 E. Triumph		Red Bobs 222	
6.8	Red Bobs 222 E. Triumph	Red Bobs 222	Preston			E. Triumph	Red Bobs 222	Red Bobs 222 E. Triumph			Huron	E. Triumph	Huron
6.7		E. Triumph Red Fife	Parker's Sel.			Red Bobs 222	E. Triumph		Huron			Huron	
6.6		Huron	Huron	E. Triumph Huron	Garnet	Huron		Huron		Huron			
6.5	Huron		Marquillo		Huron		Huron						
6.4													
6.1													
6.0													Early Prolific
5.9													
5.8				Dicklow									
5.2											Vermilion		

TABLE IX

CLASSIFICATION ON BASIS OF MINIMUM SIGNIFICANT DIFFERENCE

TEXTURE

Class	Group 1	2	3	4	5	6	7	8	9	10	11	12	13
Minimum significant difference	0.28	0.30	0.28	0.35	0.28	0.28	0.25	0.29	0.30	0.32	0.50	0.37	0.37
+1		Renfrew	Reward				Supreme						
0	Reward Marquis	Reward Marquis Kitchener	Marquis Axminster Kota Red Bobs 222 E. Triumph Preston	Reward Marquis Red Bobs 222	Reward Marquis	Hard Fed'tion Reward Marquis E. Triumph	Reward Marquis	Reward Marquis	Reward Marquis Ceres	Marquis Reward Ruby	Marquis Reward E. Triumph Red Bobs 222	Marquis Reward Pioneer	Reward Marquis E. Triumph Red Bobs 222 Brownhead
−1	Red Bobs 222 E. Triumph	Red Bobs 222 E. Triumph Red Fife Huron	Parker's Sel. Huron Marquillo	E. Triumph Huron	Red Bobs 222 E. Triumph	Red Bobs 222	Red Bobs 222 E. Triumph	Early R. Fife Red Bobs 222 E. Triumph	Red Bobs 222 Quality E. Triumph	Red Bobs 222 E. Triumph Huron	Huron	Red Bobs 222 E. Triumph Huron	Huron
−2	Huron				Garnet Huron		Huron	Huron	Huron				
−3				Dicklow									Early Prolific
−4											Vermilion		

volume. This means that the order of the varieties with respect to texture is less affected by environmental conditions than was the order with respect to loaf volume, and hence that the conclusions with regard to this characteristic will have general validity. The actual values for the averages of each group are given in Table VIII and the adjusted classification in Table IX.

These group classifications were combined on the same basis as those for loaf volume to give the single classification in Table X.

TABLE X

CLASSIFICATION OF VARIETIES ON BASIS OF TEXTURE

1. *Superior to Marquis* Renfrew, Supreme
2. *Equal to Marquis* Hard Federation Reward Marquis, Kitchener Axminster Pioneer Ceres, Kota, Ruby
3. *Slightly inferior to Marquis* Early Red Fife Red Bobs 222 Brownhead, Preston Red Fife, Quality, Early Triumph Parker's Selection Garnet Huron Marquillo
5. *Very much inferior to Marquis* Dicklow, Early Prolific Vermilion

Crumb Color

Table XI shows the difference between varieties to be significant. The interactions between variety and formula and between variety and series are low and in some of the groups are not significant. This is to be expected since the pigmentation of the flour and hence of the bread is a definite varietal characteristic. The classifications for the various groups on the basis of the actual values are given in Table XII and on the adjusted basis in Table XIII.

TABLE XI

SIGNIFICANCE OF COMPONENTS OF VARIANCE

COLOR

Group	Number of series	Ratio of Z obtained to Z at 5% point					
		Series (S)	Varieties (V)	Formulas (F)	Interactions		
					V × F	F × S	V × S
1	14	3.8	3.3	5.3	0.9	3.8	0.0
2	11	4.1	2.9	5.6	0.7	3.7	1.9
3	11	5.0	4.3	5.7	1.2	4.2	0.0
4	8	0.8	4.4	4.8	0.8	1.8	3.5
5	12	3.4	4.0	5.2	1.2	3.6	0.2
6	10	3.3	3.3	4.8	1.4	3.1	0.0
7	13	3.8	3.4	5.3	2.3	3.8	1.1
8	10	3.5	2.7	5.2	0.7	2.7	0.0
9	11	4.0	3.5	5.3	0.7	4.3	0.8
10	7	3.2	2.8	5.2	0.4	3.5	0.7
11	5	2.3	3.3	3.9	0.4	0.0	0.0
12	6	3.0	2.5	4.4	0.0	2.6	0.0
13	6	3.5	3.8	4.5	0.6	2.3	0.0

TABLE XII

AVERAGE CRUMB COLOR SCORE FOR EACH VARIETY IN EACH GROUP

Crumb color score	Group												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	Minimum significant difference												
	0.28	0.32	0.29	0.50	0.29	0.33	0.28	0.34	0.29	0.37	0.45	0.41	0.36
7.9											Marquis		
7.8											Reward		Marquis Reward
7.7									Marquis	Marquis		Marquis	
7.6	Marquis	Marquis	Marquis		Reward Marquis	Marquis	Marquis Supreme	Marquis	Reward	Reward		Reward	
7.5	Reward	Reward		Marquis		Hard Fed'tion	Reward	Reward	Quality Ceres		Red Bobs 222		
7.4		Renfrew	Reward										Red Bobs 222
7.3		Red Fife				Reward			Red Bobs 222				
7.2		Red Bobs 222	Red Bobs 222	Reward	Red Bobs 222 E. Triumph			Early R. Fife Red Bobs 222		Red Bobs 222 E. Triumph	E. Triumph	Red Bobs 222 E. Triumph	E. Triumph
7.1	Red Bobs 222 E. Triumph	E. Triumph				Red Bobs 222	Red Bobs 222 E. Triumph	E. Triumph	E. Triumph	Ruby		Pioneer	
7.0			E. Triumph	Red Bobs 222 E. Triumph		E. Triumph					Huron		Brownhead
6.9		Kitchener											
6.8			Kota										Huron
6.7		Huron	Huron Axminster			Huron		Huron	Huron	Huron			
6.6	Huron		Preston	Huron	Huron		Huron					Huron	
6.5			Marquillo Parker's Sel.										
6.4					Garnet								
6.3													Early Prolific
6.2											Vermilion		
4.7				Dicklow									

TABLE XIII

CLASSIFICATION ON BASIS OF MINIMUM SIGNIFICANT DIFFERENCE

CRUMB COLOR

Class	Group												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	Minimum significant difference												
	0.28	0.32	0.29	0.50	0.29	0.33	0.28	0.34	0.29	0.37	0.45	0.41	0.36
0	Marquis Reward Red Bobs 222 E. Triumph	Marquis Reward Renfrew Red Fife	Marquis Reward	Marquis Reward	Reward Marquis	Marquis Hard Fed'tion Reward	Marquis Supreme Reward	Marquis Reward	Marquis Reward Quality Ceres	Marquis Reward	Marquis Reward Red Bobs 222	Marquis Reward	Marquis Reward
−1		Red Bobs 222 E. Triumph	Red Bobs 222	Red Bobs 222 E. Triumph Huron	Red Bobs 222 E. Triumph	Red Bobs 222 E. Triumph	Red Bobs 222 E. Triumph	Early R. Fife Red Bobs 222 E. Triumph	Red Bobs 222	Red Bobs 222 E. Triumph Ruby	E. Triumph	Red Bobs 222 E. Triumph Pioneer	Red Bobs 222 E. Triumph
−2		Kitchener Huron	E. Triumph Kota		Huron	Huron		Huron	E. Triumph	Huron	Huron	Huron	Brownhead Huron
−3	Huron		Axminster Huron Preston Marquillo Parker's Sel.				Huron		Huron		Vermilion		
−4					Garnet								Early Prolific
−5				Dicklow									

TABLE XIV

CLASSIFICATION OF VARIETIES ON BASIS OF CRUMB COLOR

2. *Equal to Marquis*
   Marquis, Hard Federation, Supreme
   Reward
   Renfrew, Quality, Ceres
   Red Fife

3. *Slightly inferior to Marquis*
   Early Red Fife
   Red Bobs 222
   Early Triumph
   Ruby
   Pioneer

4. *Decidedly inferior to Marquis*
   Kitchener
   Kota, Brownhead, Axminster
   Huron
   Preston
   Marquillo
   Parker's Selection
   Garnet

5. *Very much inferior to Marquis*
   Vermilion, Early Prolific
   Dicklow

The combination of the individual classifications was made on the same basis as before, and is given in Table XIV.

General Appearance

The score for general appearance is the sum of the scores for shape of loaf and color of crust. The analysis of variance gave similar results to those for texture. Table XV shows that the differences between varieties are significant and while the interactions, formula × variety and series × variety, are statistically significant, they are unimportant practically. The classifications are given in Tables XVI and XVII.

The classifications by groups were combined in the same manner as for loaf volume and the combined classification is given in Table XVIII.

Absorption

The analysis of variance applied to this character differed from that applied to the others. The change was necessitated by the fact that in all laboratories the practice was followed of determining the correct absorption for each

TABLE XV

SIGNIFICANCE OF COMPONENTS OF VARIANCE

GENERAL APPEARANCE

Group	Number of series	Ratio of Z obtained to Z at 5% point					
		Series (S)	Varieties (V)	Formulas (F)	Interactions		
					V × F	F × S	V × S
1	14	3.9	2.6	3.3	1.7	4.8	1.2
2	11	3.4	3.1	3.6	2.4	4.1	0.6
3	11	3.9	2.4	3.3	1.3	5.1	1.4
4	8	2.7	4.0	3.1	2.1	2.8	0.2
5	12	3.8	2.1	2.6	1.8	4.4	1.3
6	10	3.0	1.9	3.3	1.0	3.3	1.2
7	13	3.2	2.4	3.1	1.7	4.4	1.3
8	10	4.7	3.8	4.3	3.7	6.0	4.7
9	11	3.6	2.8	3.6	1.8	5.4	0.9
10	7	3.5	2.6	2.4	1.7	4.4	0.3
11	5	2.3	4.4	2.9	3.0	1.7	0.0
12	6	2.4	1.6	2.8	1.1	2.3	0.7
13	6	2.7	3.0	3.5	1.2	2.4	1.6

TABLE XVI

AVERAGE GENERAL APPEARANCE SCORE FOR EACH VARIETY IN EACH GROUP

General appearance score	Group 1	2	3	4	5	6	7	8	9	10	11	12	13
Minimum significant difference	0.25	0.29	0.30	0.38	0.29	0.31	0.29	0.18	0.28	0.33	0.43	0.38	0.37
8.9											Marquis		
8.8											Reward	Marquis	
8.7		Reward		Marquis				Marquis	Ceres				Marquis
8.6	Reward	Renfrew Marquis		Reward		Marquis		Reward		Marquis		Pioneer Reward Red Bobs 222	Reward
8.5	Marquis		Reward Axminster Kota		Reward	Reward	Reward Marquis Supreme	Early R. Fife	Reward Marquis	Ruby Reward	Red Bobs 222		Red Bobs 222
8.4		Red Bobs 222		Red Bobs 222	Marquis	Red Bobs 222		Red Bobs 222		Red Bobs 222	E. Triumph		E. Triumph
8.3	Red Bobs 222		Marquis Red Bobs 222 Parker's Sel.		Red Bobs 222		Red Bobs 222		Red Bobs 222			E. Triumph	
8.2		Red Fife	Preston		Garnet	Hard Fed'tion							Brownhead
8.1		Kitchener							E. Triumph Quality		Huron		Huron
8.0	Huron E. Triumph	Huron	Marquillo Huron	Huron	E. Triumph	E. Triumph Huron	E. Triumph Huron	E. Triumph Huron	Huron	E. Triumph		Huron	
7.9		E. Triumph	E. Triumph	E. Triumph	Huron								
7.8										Huron			
7.7													
7.6													
7.5													Early Prolific
6.9				Dicklow									
6.2											Vermilion		

TABLE XVII

CLASSIFICATION ON BASIS OF MINIMUM SIGNIFICANT DIFFERENCE

GENERAL APPEARANCE

Class	Group												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	Minimum significant difference												
	0.25	0.30	0.30	0.38	0.29	0.31	0.29	0.18	0.28	0.33	0.43	0.38	0.37
0	Reward Marquis Red Bobs 222	Reward Marquis Renfrew Red Bobs 222	Axminster Kota Reward Red Bobs 222 Marquis Parker's Sel. Preston	Marquis Reward Red Bobs 222	Reward Marquis Red Bobs 222 Garnet	Marquis Reward Red Bobs 222	Marquis Reward Supreme Red Bobs 222	Marquis Reward	Ceres Reward Marquis Red Bobs 222	Marquis Reward Ruby Red Bobs 222	Marquis Reward	Marquis Reward Pioneer Red Bobs 222	Marquis Reward Red Bobs 222 E. Triumph
−1		Red Fife Kitchener	Hufon Marquillo E. Triumph	Huron		Hard Fed'tion Huron E. Triumph	E. Triumph Huron	Early R. Fife Red Bobs 222	E. Triumph Quality Huron	E. Triumph	Red Bobs 222 E. Triumph	E. Triumph	Brownhead Huron
−2	E. Triumph Huron	Huron E. Triumph		E. Triumph	E. Triumph Huron					Huron	Huron	Huron	
−3								E. Triumph Huron					Early Prolific
−4				Dicklow									
−5													
−6											Vermilion		

TABLE XVIII

CLASSIFICATION OF VARIETIES ON BASIS OF GENERAL APPEARANCE

2. *Equal to Marquis*
   Ceres
   Reward, Kota, Axminster
   Supreme
   Marquis, Renfrew, Ruby
   Parker's Selection, Pioneer, Preston, Garnet
   Red Bobs 222

3. *Slightly inferior to Marquis*
   Early Red Fife
   Red Fife, Hard Federation
   Kitchener
   Huron, Marquillo
   Early Triumph, Quality, Brownhead

5. *Very much inferior to Marquis*
   Dicklow, Early Prolific
   Vermilion

TABLE XIX

SIGNIFICANCE OF COMPONENTS OF VARIANCE ABSORPTION

Group	Number of series	Ratio of Z obtained to Z at 5% point	
		Varieties	Places
1	14	0.0	0.0
2	11	0.4	2.4
3	11	4.2	4.9
4	8	4.1	2.7
5	12	0.0	2.6
6	10	0.0	2.9
7	13	1.3	4.0
8	10	0.0	2.5
9	11	2.5	3.3
10	7	1.6	2.0
11	5	1.6	1.4
12	6	0.0	1.5
13	6	1.2	1 1

sample and then using the same amount of water for all subsequent bakings. The variance was therefore split into only three portions, between varieties, places and error. The results are given in Table XIX.

It will be seen that in only a few groups are the differences between varieties significant. In these experiments, only six varieties were significantly differentiated from Marquis. Kota, Ceres and Ruby had high absorption, Supreme and Brownhead were very slightly lower than Marquis, and Vermilion was decidedly low.

General Baking Quality

It is now necessary to combine the data for the individual characters into a single classification for baking quality. In the classifications given so far, once the basis was selected, the class into which each variety was placed was governed solely by the numerical data obtained. In combining these to give a classification for baking quality, however, the weight which should be given to each of the baking characteristics is a matter of opinion. It seems to us that loaf volume must be considered the most important factor because, while, in general, very large loaves are not demanded commercially, loaf volume is the most sensitive index of changes in the character of the flour and is the most susceptible of precise measurement. When the baking formula insures adequate gas production, it gives a good indication of strength, provided the flour was produced from sound wheat as in the samples here tested. It was therefore taken as the principal basis for the classification in respect to baking quality. Texture was ranked next in importance because varieties which give poor texture cannot be regarded as having good baking quality. In a study of varieties crumb color should be ranked high in importance. While loaf volume and texture are strongly influenced by environment, carotin content is essentially an inherent varietal characteristic. Although

yellow color in flour can be bleached quite readily, good color is desirable since bleaching is prohibited in some countries, adds to the expense of flour manufacture, and may result in damage to the baking quality if an overdosage of bleaching agent is used. The general appearance of the loaf is intimately connected with loaf volume and generally a loaf which is satisfactory in other respects will be satisfactory in appearance. High absorption is a characteristic greatly desired by bakers. However, our data show that the amount of water that a flour will absorb depends more on the environmental conditions under which the wheat is grown than on the variety. There are a few varieties, however, which vary from the normal and account of this is taken in the classification.

The final arrangement of the varieties in the order of relative baking quality was made by combining the single character classifications. Varieties with very good texture were raised above the class in which their loaf volume would have placed them. Varieties were not raised for excellence in color and appearance but were lowered if these characters were markedly faulty. Absorption had to be considered in only a few cases. Varieties which fell into Class 4 (decidedly inferior to Marquis) for any single baking characteristic were never placed higher than Class 3 (slightly inferior to Marquis) in the final classification. This was done because it was considered that a variety should not be placed in the same group with Marquis if it had any marked faults, since one such fault may be sufficient to condemn a variety commercially.

The results of this classification are given in Table XXI.

TABLE XX

Classification of varieties on basis of absorption

1. *Superior to Marquis* Kota, Ceres, Ruby
2. *Equal to Marquis* Axminster, Dicklow, Early Prolific, Early Red Fife, Early Triumph, Garnet, Hard Federation, Huron, Kitchener, Marquillo, Marquis, Parker's Selection, Pioneer, Preston, Quality, Red Bobs 222, Red Fife, Renfrew, Reward
3. *Slightly inferior to Marquis* Supreme Brownhead
5. *Very much inferior to Marquis* Vermilion

TABLE XXI

Classification of varieties on the basis of general baking quality

1. *Superior to Marquis* Reward Ceres
2. *Equal to Marquis* Marquis Pioneer, Supreme, Renfrew
3. *Slightly inferior to Marquis* Red Fife, Red Bobs 222, Early Red Fife Ruby, Axminster, Quality, Kota Early Triumph
4. *Decidedly inferior to Marquis* Kitchener, Preston, Marquillo, Parker's Selection Garnet, Huron, Brownhead Hard Federation
5. *Very much inferior to Marquis* Dicklow, Vermilion, Early Prolific

Milling Quality

In the study of milling quality the yield of straight grade flour and the milling properties were considered. The average flour yields obtained by the three laboratories for each sample were submitted to a variance analysis. In order to eliminate known causes of variability, the variance between years was calculated, thus making it possible to secure the three interactions, variety and place, variety and year, place and year. This made it possible to remove the significant interactions from error.

The significance of the various portions of the variance is given in Table XXII. It will be seen that the differences between the varieties in Group 3 are not significant. The interaction of variety with place is significant in only three groups and the interaction of variety and year is not significant in any of the groups. This means that the relative order of the varieties with respect to flour yield is little affected by the environment. The average results for each group are given in summary form in Table XXIII and reclassified on the basis of the significant difference in Table XXIV. Since the differences between the varieties in Group 3 are not significant, no value for the minimum significant difference can be given.

TABLE XXII

Significance of components of variance
Yield of straight flour

Group	Number of series	Ratio of Z obtained to Z at 5% point					
		Varieties (V)	Places (P)	Years (Y)	Interactions		
					V × P	V × Y	P × Y
1	16	1.3	2.4	1.5	0.0	0.0	1.9
2	16	1.1	2.3	0.0	0.6	0.0	2.6
3	14	0.7	2.2	0.0	0.0	0.0	2.7
4	12	1.7	2.0	0.6	0.0	0.0	2.8
5	8	1.9	2.2	0.6	1.2	0.6	0.9
6	8	3.8	2.9	1.8	0.1	0.0	1.9
7	6	1.8	0.0	1.4	1.0	0.0	2.1
8	12	3.4	2.5	0.0	1.2	0.2	2.2
9	12	2.9	2.0	0.0	0.1	0.2	1.6
10	14	1.2	2.6	0.9	0.0	0.0	1.8
11	6	3.3	2.9	—	—	—	—
12	5	2.9	2.7	—	—	—	—

The information in Tables XXIII and XXIV was condensed to give the classification on the basis of flour yield shown in Table XXV.

It was pointed out previously that flour yield is not the only factor in milling quality. Another classification is given in Table XXVI, which takes these other factors into account. Only a few of the varieties are abnormal in their milling or tempering behavior. Kota resembles the durums in its milling characteristics. The middlings are difficult to reduce and consequently the power required is greater than for normal varieties. Garnet requires longer tempering than the normal varieties and the middlings are more difficult to

TABLE XXIII

YIELD* OF STRAIGHT FLOUR FOR EACH VARIETY IN EACH GROUP

Yield of straight flour, %	Group 1	2	3	4	5	6	7	8	9	10	11	12
	Minimum significant difference (%)											
	0.88	1.07	—	1.62	1.09	1.16	1.15	0.86	1.10	0.82	2.74	3.10
69.9 to 69.5						Marquillo	E. Triumph	Marquillo	Parker's Sel.			
69.4 to 69.0							Red Bobs 222				Quality	Quality
68.9 to 68.5						Red Fife E. Triumph						
68.4 to 68.0					Quality	Preston Red Bobs 222	Red Fife Supreme					
67.9 to 67.5	Red Fife	Red Bobs 222	E. Triumph			Kota Supreme	Garnet Reward Huron Ruby	Kota		Red Fife Supreme		
67.4 to 67.0	Supreme		Kota Preston	E. Triumph Kota Axminster Preston Huron	Reward Ceres	Huron Kitchener	Kitchener Marquis		Axminster		Kota	
66.9 to 66.5	Reward Kitchener Marquis	Marquis Supreme Reward	Huron		Marquis	Marquis Garnet Reward	Renfrew	Reward	Kota	Reward Kitchener Marquis	Preston Axminster E. Triumph Ceres	Axminster Kota
66.4 to 66.0	Renfrew		Marquis Supreme Reward	Marquis	Garnet Renfrew	Renfrew		Garnet Marquis	Reward Marquis	Early R. Fife Renfrew	Huron Reward	Preston E. Triumph Ceres Pioneer
65.9 to 65.5				Reward Supreme				Renfrew			Marquis Supreme	Reward Marquis Huron
65.4 to 65.0									Renfrew		Early Prolific	Supreme
64.9 to 64.5				Hard Fed'tion							Brownhead	Early Prolific
64.4 to 64.0											Hard Fed'tion	Hard Fed'tion Brownhead
63.9 to 63.5						Dicklow						
61.4 to 61.0											Vermilion	
60.9 to 60.5												Vermilion

TABLE XXIV
CLASSIFICATION ON BASIS OF MINIMUM SIGNIFICANT DIFFERENCE
YIELD OF STRAIGHT FLOUR

Class	Group											
	1	2	3	4	5	6	7	8	9	10	11	12
	Minimum significant difference											
	0.88	1.07	—	1.62	1.09	1.16	1.15	0.86	1.10	0.82	2.74	3.10
+3								Marquillo	Parker's Sel.			
+2	Red Fife					Marquillo						
+1					Quality	Red Fife E. Triumph Preston Red Bobs 222	E. Triumph Red Bobs 222	Kota	Axminster	Red Fife Supreme	Quality	Quality
0	Supreme Reward Kitchener Marquis Renfrew	Red Bobs 222 Marquis Supreme Reward	E. Triumph Kota Preston Huron Marquis Supreme Reward	E. Triumph Axminster Kota Huron Marquis Reward Supreme	Reward Ceres Marquis Garnet Renfrew	Kota Supreme Huron Kitchener Marquis Garnet Reward Renfrew	Red Fife Supreme Garnet Reward Huron Ruby Kitchener Marquis Renfrew	Reward Garnet Marquis Renfrew	Reward Marquis Renfrew	Reward Kitchener Marquis Early R. Fife Renfrew	Kota Preston Axminster E. Triumph Ceres Huron Reward Marquis Supreme Early Prolific Brownhead Hard Fed'tion	Axminster Kota Preston E. Triumph Ceres Pioneer Reward Marquis Huron Supreme Early Prolific Hard Fed'tion Brownhead
−1				Hard Fed'tion							Vermilion	Vermilion
−2						Dicklow						

TABLE XXV

Classification of varieties on basis of yield of straight flour

1. *Superior to Marquis*
Marquillo, Parker's Selection
Early Triumph, Red Bobs 222, Red Fife, Quality
2. *Equal to Marquis*
Kota, Preston, Huron, Ruby, Supreme, Axminster
Reward, Marquis, Kitchener, Garnet, Ceres
Renfrew, Pioneer, Early Red Fife
3. *Slightly inferior to Marquis*
Brownhead, Early Prolific, Hard Federation
5. *Very much inferior to Marquis*
Vermilion, Dicklow

TABLE XXVI

Classification of varieties on basis of milling quality

1. *Superior to Marquis*
Marquillo, Parker's Selection
Early Triumph, Red Bobs 222, Red Fi Quality
2. *Equal to Marquis*
Preston, Huron, Ruby, Supreme, Axminster
Reward, Marquis, Kitchener, Ceres
Renfrew, Pioneer, Early Red Fife
3. *Slightly inferior to Marquis*
Kota, Garnet
Brownhead, Early Prolific, Hard Federation
5. *Very much inferior to Marquis*
Vermilion, Dicklow

reduce, though not so difficult as those of Kota. Vermilion, Hard Federation and Dicklow have a tendency to flake rather than to grind. However, this is reflected in the low yield of straight flour and these varieties need not be further discounted on this account. The classification is given in Table XXVI.

Milling and Baking Quality

In order to arrive at a classification for milling and baking quality the classifications for milling quality and baking quality were combined. In doing this the baking quality was taken as the basis and the placing of the varieties was modified in accordance with the milling quality. Varieties with baking quality only slightly inferior to Marquis were raised if the milling quality was high. Varieties with decidedly inferior baking quality were not raised on account of high milling quality because serious defects in baking are sufficient to discredit a variety commercially. Low milling quality lowered the placing. This method of classification places the emphasis on baking quality and was adopted because in the export market high baking quality is the prime requisite in our wheats. At the same time it takes the milling quality very definitely into account. The classification is given in Table XXVII.

The first class includes varieties of excellent or good quality. If Canada's wheat crop could be restricted to these eight varieties there would be a substantial improvement in its general quality. It is fortunate that this class includes varieties with a wide range of agronomic characteristics so that there is little reason for a farmer going outside of this group in selecting a variety.

The second class includes varieties of fair quality. Unless these varieties made up a very large percentage of our crop no great harm would be done to its general quality.

TABLE XXVII

CLASSIFICATION OF VARIETIES ON THE BASIS OF SUITABILITY FOR EXPORT AND DOMESTIC MILLING

1. Varieties which are similar to Marquis in milling characteristics and superior to, equal to or only slightly inferior to, Marquis in milling and baking quality. These varieties may be considered satisfactory for export and domestic milling.
   Reward, Ceres, Marquis, Pioneer, Red Fife, Renfrew, Red Bobs 222, Supreme

2. Varieties which are similar to Marquis in milling characteristics, but which are inferior to Marquis in baking quality. These varieties may be present in a fair percentage in a mill mix without seriously affecting the quality.
   Early Red Fife, Ruby, Early Triumph

3. Varieties which differ markedly from Marquis in color and shape of kernel, in milling characteristics, or are so decidedly inferior in baking quality as to depreciate seriously the commercial value of export shipments.
   (a) White wheats
       Axminster, Quality, Hard Federation
   (b) Varieties differing from Marquis in milling characteristics.
       Garnet, Kota
   (c) Varieties inferior to Marquis in baking characteristics.
       Garnet, Parker's Selection, Brownhead, Huron, Kitchener, Preston, Marquillo

4. Varieties so decidedly inferior to Marquis in milling and baking quality as to depreciate seriously the quality of export cargoes when present in any appreciable percentage.
   Early Prolific, Dicklow, Vermilion

The third class includes varieties of poor quality that have been discounted for various causes. They are all objectionable in several respects. Their presence in any appreciable quantity in a cargo of wheat is bound to reduce the average quality. In view of this fact, these varieties should not be grown in western Canada. Measures should be taken to discourage the use of those that are extensively grown, whatever their agronomic characteristics may be.

It will be observed that the white wheats have been classified as unsatisfactory without regard to their placings in Tables XXI and XXVI. The presence of these varieties in our export wheat is objectionable whatever their milling and baking quality, because they cannot easily be distinguished from starchy red wheat. The quantity of white wheat grown is, and probably always will be, small. The difficulty of merchandizing small lots of wheat makes it undesirable that white wheat should be grown in western Canada, except to fill the very limited demand of biscuit flour millers. Even this is somewhat objectionable because of the almost inevitable mixing with red wheats on the farm.

The fourth class includes varieties which are decidedly bad in quality. The growing of these varieties should be actively discouraged.

PROTEIN CONTENT AND WEIGHT PER BUSHEL

The protein content and weight per bushel of the varieties under study were investigated. The results were not used in the classification of the varieties for milling and baking. These characters partly determine the milling and baking quality and their effect in this regard is covered by the results of the milling and baking tests. However, because of their wide use as

indices of milling and baking quality, classifications based on them are included. These classifications were arrived at by the examination of the results of the analysis of variance of several groups of varieties. The procedure was similar to that employed in the study of the milling and baking data. As these characters are of secondary importance, only the final classifications are given.

TABLE XXVIII

CLASSIFICATION OF VARIETIES ON BASIS OF PROTEIN CONTENT

1. *Superior to Marquis* Reward Kota Ceres, Ruby, Early Prolific
2. *Equal to Marquis* Red Fife Marquis, Marquillo, Parker's Selection, Vermilion Pioneer, Brownhead, Preston, Axminster, Quality
3. *Slightly inferior to Marquis* Garnet Huron Early Triumph Red Bobs 222, Renfrew, Early Red Fife Supreme, Hard Federation Kitchener
5. *Very much inferior to Marquis* Dicklow

TABLE XXIX

CLASSIFICATION OF VARIETIES ON BASIS OF WEIGHT PER MEASURED BUSHEL

1. *Superior to Marquis* Reward Kota
2. *Equal to Marquis* Hard Federation, Axminster, Ruby, Supreme, Parker's Selection, Ceres, Quality Marquis, Preston, Pioneer, Brownhead, Early Prolific Huron, Red Bobs 222, Kitchener, Early Red Fife, Early Triumph, Garnet
3. *Slightly inferior to Marquis* Red Fife, Renfrew, Marquillo
5. *Very much inferior to Marquis* Dicklow, Vermilion

Description of Varieties

In the foregoing sections of this paper discussion of the characteristics of individual varieties has been omitted. In this section the milling and baking characteristics of each variety will be discussed and the reasons for placing certain of the varieties in a particular class in the classification pointed out. The varieties are arranged in classes and in alphabetical order within each class. It should be pointed out that the classification is based wholly on milling and baking quality, and that the practical utility of certain varieties will be limited by their agronomic characteristics.

1. *Varieties which are satisfactory for export and domestic milling*

Ceres.—This variety has satisfactory weight per bushel and a high protein content. The flour yield is equal to Marquis. It has excellent baking properties, giving a loaf of large volume and satisfactory characteristics by all formulas. The absorption is high.

Marquis.—Marquis is our standard variety and is satisfactory in all respects.

Pioneer.—This variety is similar to Marquis in weight per bushel and protein content. It gives a satisfactory yield of flour. It slightly excels Marquis in loaf volume when baked using the simple or bromate formulas. However it is not quite equal to Marquis in color of crumb.

Red Bobs 222.—Red Bobs 222 has a satisfactory test weight but only fair protein content. It gives an excellent yield of flour. The loaf volume is equal to Marquis except when the bromate formula is used and then the volume is only slightly lower. The loaves are slightly poorer in color than those of Marquis. The texture is poorer than that of Marquis when the malt-phosphate formula is used. On the whole this variety is not equal to Marquis in baking quality but its excellent flour yield entitles it to a place in this class.

Red Fife.—This variety is rather peculiar in that it has a low weight per bushel but an excellent flour yield. The protein content is satisfactory. The loaf volume is not significantly different from that of Marquis on the average. The simple formula gives a slightly lower volume. It is not equal to Marquis in texture and general appearance. Its baking quality is not as good as that of Marquis. In spite of this it is placed in this class because of its excellent flour yield. It should be pointed out that the reputation of this variety for good baking quality can probably be accounted for in part by the fact that it is late in maturing and hence its use is confined to the high quality areas.

Renfrew.—Renfrew has only moderate weight per bushel but a satisfactory flour yield. It is rather low in protein content but it is satisfactory in its baking qualities. It is equal to Marquis in loaf volume when baked with the simple or malt-phosphate formulas but it is deficient in this respect when baked with formulas containing potassium bromate. However, the texture by all methods is superior to that of Marquis and the color, appearance and absorption are satisfactory. On the whole this variety has slightly lower quality than Marquis but the difference is not sufficient to warrant placing it in a different class.

Reward.—Reward has a high weight per bushel and a satisfactory flour yield. It has a very high protein content. The baking qualities are excellent. It gives loaves of large volume with good color, texture, appearance and absorption. Reward has the best milling and baking quality of the varieties tested.

Supreme.—This variety has satisfactory test weight and milling quality. The protein content is lower than that of Marquis. It is only moderately satisfactory in baking quality. The loaf volume is lower than that of Marquis when the bromate formula is used. The texture, however, is excellent and the color and appearance are satisfactory. Supreme is slightly deficient in absorption. This variety has the poorest quality in this class.

2. *Varieties which are slightly inferior to Marquis but which may be present in a fair percentage in a mill mix without seriously affecting the quality*

Early Red Fife.—This variety has satisfactory weight per bushel and yield of flour. Its protein content is low. The absorption is satisfactory but in all the other baking characteristics it is only fair.

Early Triumph.—Early Triumph has a satisfactory weight per bushel and an excellent flour yield. Its protein content is low. The absorption is satisfactory but it is deficient in the other baking characteristics. The loaf volume and the color are not equal to Marquis and the texture and general appearance are poor. The baking quality is too low to warrant placing this variety in the first class even though the milling quality is excellent.

Ruby.—Ruby has a satisfactory test weight and flour yield. It has a high protein content and high absorption. It is only fair in loaf volume, texture and color. This variety has no outstanding defects but its quality is not sufficiently high to warrant placing it in the first class.

3. *Varieties which are unsatisfactory for export or domestic milling*

a. White Wheats

Axminster.—This variety has good test weight and milling yield. It is fair in protein content. The baking characteristics are good with the exception of color, the loaves being rather yellow.

Hard Federation.—Hard Federation has good weight per bushel but gives a low yield of flour. It is fair in protein content. It gives small loaves of rather poor appearance. The other baking characteristics are satisfactory.

Quality.—The milling quality is good. The weight per bushel and protein content are similar to those of Axminster. The loaf volume, texture and appearance are fair and the color of the crumb is good.

b. Red Wheats

Brownhead.—Brownhead is satisfactory in weight per bushel but it gives a low yield of flour. It is low in protein. The absorption is fairly satisfactory and the texture and appearance of the loaves are fair but the other baking characteristics are poor.

Garnet.—The test weight and the yield of flour are satisfactory. Garnet differs from Marquis in its tempering properties and cannot be tempered properly when mixed with that variety. For this reason the milling quality of Garnet is classed as fair. The protein content is low. It is satisfactory in absorption and in appearance of the loaves. The other baking characteristics are poor. It gives small loaves with poor texture, particularly when baked by the blend-bromate or malt-phosphate formulas. The color of the crumb is decidedly yellow. Garnet cannot be considered a desirable variety.

Huron.—The weight per bushel and the milling yield are satisfactory, but Huron is poor in all the baking characteristics with the exception of absorption.

Kitchener.—Kitchener has normal test weight and flour yield. The protein content is low. Absorption and texture are satisfactory. The appearance is fair and the loaf volume and color are decidedly poor.

Kota.—Kota has a high test weight and a satisfactory flour yield. However, since the endosperm is durum-like in character it does not mill as easily as the ordinary spring wheats. For this reason, its milling quality can only be classed as fair. It is excellent in protein content, absorption and loaf volume and the texture and appearance are satisfactory. However, the crumb has a pronounced yellow color which is objectionable. This variety has several excellent characteristics and two serious defects. It is placed in this class on account of the difficulty of milling and the color of the bread.

Marquillo.—Marquillo resembles Red Fife in that it has a low weight per bushel but an excellent milling yield. The protein content, absorption and loaf volume are satisfactory but the other baking characteristics, particularly the crumb color, are poor. In spite of the high flour yield the general quality is unsatisfactory.

Parker's Selection.—This variety, sometimes known as Parker's Marquis, is satisfactory in test weight and gives a high yield of flour. It has a normal protein content and gives a loaf which is satisfactory in absorption, volume and appearance. The texture and the color are too poor, however, to permit a higher placing. In addition, it was noticed that the handling qualities of the dough of this variety were particularly poor.

Preston.—The weight per bushel and flour yield are satisfactory. The protein content is low. The loaves have good appearance and fair volume and texture but the crumb color is poor.

4. *Varieties which are very unsatisfactory for export or domestic milling*

Dicklow.—Dicklow is low in weight per bushel and flour yield. It is very low in protein content and very poor in all the baking characteristics.

Early Prolific.—This variety has fair weight per bushel and fair milling yield. The loaf volume is poor and all the other baking characteristics are very poor.

Vermilion.—Vermilion has a fair protein content but in all other respects it can only be classed as "bad".

Acknowledgments

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References

1. Associate Committee on Grain Research, National Research Council of Canada. Collected Papers, Vol. I. 1932.
2. Fisher, R. A. Statistical Methods for Research Workers. Edinburgh, Oliver and Boyd. 1928.
3. Geddes, W. F., Malloch, J. G. and Larmour, R. K. The milling and baking quality of frosted wheat of the 1928 crop. Can. J. Research, 6: 119-155. 1932.
4. Stansfield, E. and Cook, W. H. The Drying of Wheat (Second Report). Report No. 25. National Research Council of Canada. 1932.

STUDIES ON THE FAILURE OF HYBRID GERM CELLS TO FUNCTION IN WHEAT SPECIES CROSSES[1]

BY W. P. THOMPSON[2] AND J. M. ARMSTRONG[3]

Abstract

Chromosome numbers were determined in numerous male gametophytes of F_1 between 21- and 14-chromosome species of wheat. The results show that pollen grains with various chromosome numbers from 14 to 21 are actually formed and in approximately the theoretically expected proportions. The lack of plants in later generations which should result from the functioning of pollen grains with intermediate numbers is therefore not due to the failure of such grains to be formed because of a lack of random segregation at the second reduction division.

Grains with intermediate numbers are retarded in their nuclear development, so that counts made on stamens in which division is most active give a smaller proportion of grains with intermediate numbers and a higher proportion with parental numbers than is expected theoretically. Retardation in nuclear development is correlated with a deficiency in cytoplasmic contents, 10 to 15% of the grains showing little or no cytoplasm, and another 15 or 20% showing some degree of reduction in cytoplasm. All grains with reduced cytoplasm and some of those with normal contents are so retarded in nuclear development (having only one or two nuclei or no organized male cells) that they could not function when the normal ones are mature and the stamen dehisces. Unfavorable chromosome conditions in grains with intermediate numbers cause a complete abortion of some grains and retardation of nuclear development in others.

Under the best available experimental conditions only 11 or 12% of F_1 pollen grains germinate, in contrast to 70 or 80% for parental pollen. No grains with reduced cytoplasm germinate, and at least 50% of those with apparently normal cytoplasm fail to germinate.

Introduction

It has been shown by Thompson and Cameron (2) and by Sax (1) that in F_1 between 21- and 14-chromosome species of wheat nearly all the male gametes which produce viable offspring have the parental chromosome numbers, or only one more or less. But to judge by events at the meiotic divisions, the great majority of the pollen grains should have intermediate chromosome numbers. Therefore nearly all those with intermediate numbers must fail to produce offspring. The great difference between the proportions of expected and functioning grains with the different chromosome numbers is illustrated graphically in Fig. 1. The female gametes with intermediate numbers are much more successful than the male.

There are several possible causes for the failure of gametes with intermediate numbers to produce viable offspring. The present paper gives the results of an attempt to determine whether certain possible causes actually have any effect, and if so, how important the effect is. The first possibility investigated is that gametes with intermediate numbers may not actually be formed in expected proportions owing to a lack of random segregation at the second reduction division. If there should be a tendency for all the 7 univalent *vulgare* chromosomes to go to one pole, the resulting pollen grains would tend to have 14 + 7 or 14 + 0 chromosomes. This would account for the results

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to be explained. The movement of the univalents seems to be a random one as observed microscopically. But hitherto no attempt has been made to determine the numbers in the pollen grains and thereby to decide whether the segregation actually is random.

The second possibility investigated is that, owing to unfavorable chromosome conditions, those with intermediate numbers may be retarded in development, or for other reasons unable to function when the others do. It is well known, of course, that 10 to 15% of the pollen is visibly abortive, but this proportion is not nearly high enough to account for the results. The apparently good pollen was therefore investigated.

Frequencies of Different Chromosome Numbers in F_1 Pollen

Methods and Materials

The chromosome numbers in the individual pollen grains were determined directly by the study of the divisions in the male gametophyte. In wheat both gametophytic divisions take place before the pollen is shed. The first produces the tube and generative cell and the second the two sperm cells from the generative cell. Mature pollen contains two well-organized, elongated male gametes.

All the counts recorded were made at the first division because the second is very difficult to study. By the time it occurs a large amount of starch has accumulated and confined the chromosomes to a compact mass. It would have been desirable to determine whether pollen of certain chromosome types fails to accomplish the second division, but, though efforts were made to do so, reliable counts were too difficult to make in sufficient numbers.

The chief difficulty in studying the first division was with the fixation, owing to the thick, hard wall of the grain and to the presence of a large vacuole. With care, satisfactory fixation was accomplished both in Nawaschin's solution and in Allen's modification of Bouin's fluid. It was necessary to remove the stamens from the flowers, to fix them alone without other floral parts, and to subject them to the suction of an air pump during a preliminary period of one or two minutes in a mixture of three parts of absolute alcohol and one of glacial acetic acid.

The plants used were hybrids between the *vulgare* variety Marquis and *dicoccum* (Vernal), *durum* (Medeah), and *persicum* (Black Persian).

Results

The counts on 189 grains from anthers in which division was proceeding actively are given in Table I, together with those to be expected on the basis of random segregation of the univalents at the second reduction division, and with those for functioning grains obtained by Thompson and Cameron by the study of plants of the next generation. In regard to 133 of the 189 grains we are quite sure of the counts; the other 56 are correct within the limits ± 1, and we believe they are fully correct. It is considered desirable to record every grain which is countable in the preparations used, in order to avoid the possibility that the ones which are most difficult to count and which might belong to

certain chromosome types are being omitted. In addition to the 189 there is a group of 37 on the same slides, in which the exact number could not be determined but which could be placed in three classes; (a) with 14 or 15, (b) with 16 to 19, (c) with 20 or 21. The frequency distribution of these is not different from that of corresponding groups recorded in Table I.

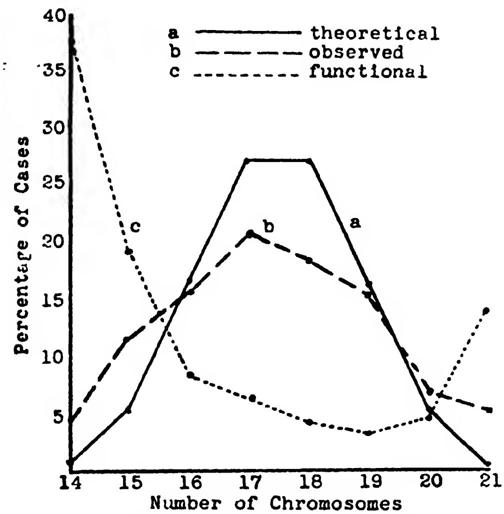

FIG. 1. *Frequency distribution of chromosome numbers in F_1 pollen grains; a, theoretical; b, observed; c, functional.*

It is plain that the actual frequency of the different chromosome numbers is, in a general way, similar to that which would be expected if the seven univalent *vulgare* chromosomes move at random to either pole at the second reduction division. It is very different, however, from that of the pollen grains which function successfully (or at least produce viable offspring) as determined by Thompson and Cameron and by Sax. These points are illustrated graphically in Fig. 1. The curve for the actual counts (b) is of the same general form as that expected (a), but the curve for the functional grains is inverted (c). These curves show that all chromosome types of pollen grains are actually produced approximately in the proportions expected on the basis of random segregation, but that the great majority of those with intermediate numbers do not function, or at any rate, do not produce viable offspring.

TABLE I

FREQUENCIES OF CHROMOSOME NUMBERS IN POLLEN GRAINS OF F_1

Cross	Chromosome number								Totals
	14	15	16	17	18	19	20	21	
vulgare × dicoccum	3	6	14	15	9	12	4	3	61
vulgare × durum	1	7	7	9	8	4	2	1	39
vulgare × persicum	4	9	8	15	19 .	15	7	7	84
Totals	8	22	29	39	36	31	13	11	
Percentages	4.2	11.7	15.3	20.7	19.0	16.4	6.9	5.8	189
Expected	0.8	5.5	16.4	27.3	27.3	16.4	5.5	0.8	
Functional	37 7	19.3	8.8	7.0	4.4	3 5	5.2	14.0	

Although there is a general similarity between the actual and expected results, nevertheless there is a noticeable deficiency of grains with intermediate numbers and an excess of those with extreme numbers (14 and 15, 20 and 21)

recorded in Table I. Curve (b) of Fig. 1 begins and ends at a higher level than curve (a), but does not rise as high. The expected ratio of grains with the extreme numbers 14, 15, 20 and 21, to those with the intermediate numbers 16, 17, 18 and 19, is 1 to 7.5. The actual ratio is 1 to 2.5.

It was considered possible that grains with intermediate numbers might appear to be deficient because they might develop slowly owing to their unfavorable chromosome combinations. The spread in the time of division among different grains of the same pollen sac is much greater than among pollen mother cells. The counts recorded in Table I were made on stamens in which a considerable proportion of the grains were actively dividing. The grains which were late in dividing owing to unfavorable chromosome conditions would be missed from such counts. In order to test this possibility stamens were studied in which nearly all the grains had divided (were already binucleate). Chromosome counts were made on the few that were still dividing. The frequencies of the chromosome numbers in these retarded grains are shown in Table II.

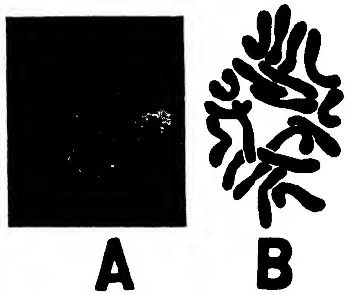

FIG. 2. *A. Photomicrograph of first gametophytic division in a pollen grain with 19 chromosomes. ×900. B. The same; camera lucida drawing.*

TABLE II

FREQUENCIES OF CHROMOSOME NUMBERS IN F_1 GRAINS WHICH WERE RETARDED IN DEVELOPMENT

Chromosome numbers	14	15	16	17	18	19	20	21	Total
Frequency		1	5	12	14	6	2		40

It is plain from this table that the grains which develop slowly usually have intermediate numbers. Apparently the chromosome conditions in such grains are unfavorable for vigorous development. The deficiency of intermediate numbers in counts made when division figures are abundant would therefore be explained. It will be shown later that many of the tardy grains never reach the stage of the first division but remain uninucleate and that others which pass the first division fail to reach the second or to form sperms.

Another general feature of the results is that there are more grains in the four lowest number classes (with 14 to 17 chromosomes) than in the four highest (18 to 21). This is particularly striking in *vulgare* × *dicoccum* and *vulgare* × *durum*. The cause is doubtless the loss of lagging univalent chromosomes which fail to reach the poles at the homotypic division in time to be included in the daughter nuclei. This would cause a general reduction in chromosome numbers (Watkins (4), Thompson and Hollingshead (3)).

Relation Between Cytoplasmic Content and Nuclear Development of Pollen Grains

It was shown in the previous section that there is great variation in the rate of early development of the pollen grains and that the slower grains have intermediate numbers. It is therefore desirable to trace the later development and ultimate fate of such grains.

Methods

Stamens at various stages between the completion of the meiotic divisions and maturity were fixed as previously described and preserved in 70% alcohol. The examination was made in smear preparations in aceto-carmine. This made possible the study both of cytoplasmic and nuclear conditions in well-fixed grains at all stages of development. Caution is necessary in the study of mature grains by this method because in some of them the wall bursts and is likely to be thrown off and appear as an empty grain.

Development of Pollen Grains

(a) *Normal*

Following the meiotic divisions the young pollen grains contain a rather dense cytoplasm with no vacuoles. Then follows a period of rapid enlargement which is not accompanied by a corresponding increase in the amount of cytoplasm. The result is the appearance of a large vacuole which occupies most of the space in the young grain, the cytoplasm being confined to a thin layer at the periphery (Fig. 3, A). In normally developing grains the vacuole then gradually becomes filled with cytoplasm. As soon as this process is completed, starch is deposited and eventually is present in a dense mass.

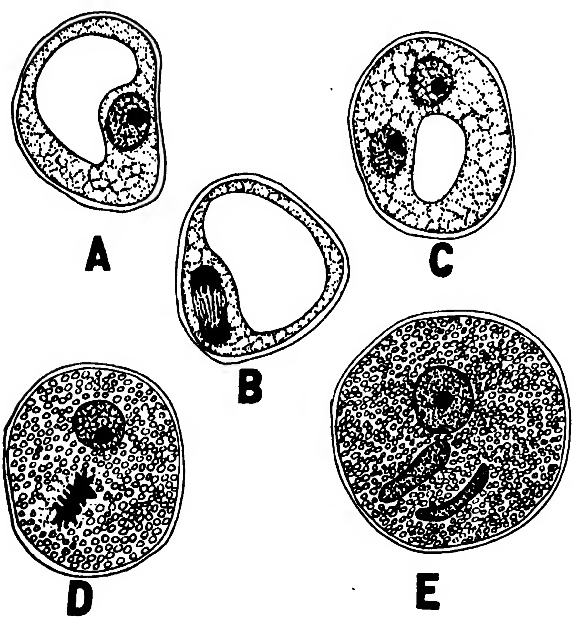

FIG. 3. *Different stages in the normal development of a pollen grain of wheat (see text).*

The first gametophytic division, which forms the tube and generative cells, takes place while the vacuole is still of considerable size (Fig. 3, B). The second division occurs after the vacuole has been filled and a large amount of starch has been deposited (Fig. 3, D). The two sperm nuclei which result from this division only gradually assume the characteristic elongated form (Fig. 3, E).

In most species and hybrids the first division takes place in the oldest flower of the oldest spikelets at the time the spike is pushing through the sheath. Periods of about two days elapse between the first and second divisions and between the second division and the maturity of the pollen. The spikelets at the tip and base of the spike lag a day or so behind those at the centre in respect to all these events.

(b) *Abnormal*

In the F_1 all pollen grains appear to develop normally until the large vacuole is present. In many of them the development then becomes abnormal in that the cytoplasm does not increase sufficiently to fill the vacuole. In some the cytoplasm not only fails to increase but degenerates at an early stage, so that it has disappeared by the time that those which develop normally have reached the stage of the first division. In others the cytoplasm increases very slowly; these lag further and further behind the normal ones. In different grains there are various rates of cytoplasmic increase and many apparently cease developing entirely at various stages. Consequently in mature stamens various amounts of cytoplasm may be observed in different grains, from the normal dense mass to none at all.

The grains in anthers at certain stages of development were classified according to their cytoplasmic content into four groups; those with normal, reduced, slight, and no cytoplasm. The percentages of grains in these four classes at the stage when the first gametophytic division is most abundant are given in Table III. Even in pure species there is a small percentage of grains with less than the normal amount of cytoplasm, but in the hybrids the percentage is much higher. It is notable that the sum of the percentages in the classes "slight" and "none" is nearly equal to the percentage of visibly aborted mature pollen as reported by several authors. Evidently most of the grains that will completely abort can be recognized at this stage.

TABLE III

PERCENTAGES OF GRAINS WITH VARIOUS AMOUNTS OF CYTOPLASM IN ANTHERS IN WHICH THE FIRST GAMETOPHYTIC DIVISION IS TAKING PLACE

Material	Cytoplasmic condition				Total
	Normal	Reduced	Slight	None	
vulgare	92.8	4.8	1.6	0.8	842
dicoccum	82.6	13.4	3.2	0.8	522
persicum	88.9	9.1	1.7	0.3	736
F_1 vulgare × dicoccum	56.1	33.0	9.3	1.6	1653
F_1 vulgare × durum	58.1	27.7	12.8	1.4	1343
F_1 vulgare × persicum	66.6	24.9	6.6	1.9	1350

NUCLEAR CONDITIONS IN VARIOUS POLLEN TYPES

In the previous reports regarding abnormal or abortive pollen in crosses of this type, no account has been taken of nuclear conditions. When methods are used which reveal the condition of the nuclei as well as that of the cytoplasm,

a strong correlation becomes evident between the amount of cytoplasm and the degree of nuclear development, even where the reduction in the amount of cytoplasm is small. This is shown in the accompanying tables.

TABLE IV

PERCENTAGE OF F_1 POLLEN GRAINS OF VARIOUS CYTOPLASMIC TYPES THAT WERE *uninucleate* (REST BINUCLEATE) WHEN SOME WERE UNDERGOING FIRST GAMETOPHYTIC DIVISION

Material	Cytoplasmic condition			
	Normal	Reduced	Slight	None
vulgare × dicoccum				
stamen 1	11 2	35.9	90.5	100.0
stamen 2	52 2	61.2	89.0	100.0
stamen 3	22.5	47 9	88.2	100.0
vulgare × durum				
stamen 1	19.7	43.9	85 7	100.0
stamen 2	9.5	43.2	89.4	100.0
stamen 3	5.9	33 6	91.2	100 0

The data in Table IV were collected from stamens in which many of the grains were undergoing the first division. It shows the percentage of grains in each of the four cytoplasmic classes which were uninucleate at that time, the rest being binucleate. The stamens are recorded individually since the percentage of uninucleate grains decreases with the development of the stamen. It is plain that the less cytoplasm there is in a grain, the more retarded is the nuclear development. None of those which lacked cytoplasm and only a small percentage with a "slight" amount had divided, whereas the great majority of those with a normal amount had done so.

Similar results were obtained at a later stage when the second division was occurring in some of the grains. Table V gives the results for one stamen which was typical of many studied. Those in the class "three nuclei" were ones in which the generative nucleus had divided but in which the daughter nuclei had not yet been organized as sperms. The majority of those with normal cytoplasm already possessed sperms and nearly all had divided twice; but few of

TABLE V

RELATION BETWEEN NUCLEAR DEVELOPMENT AND CYTOPLASMIC CONDITION IN F_1 POLLEN WHEN SECOND GAMETOPHYTIC DIVISION IS OCCURRING (VULGARE × PERSICUM)

Nuclei	Cytoplasm			
	Normal	Reduced	Slight	None
Sperms present	56.8	1.0		
Three nuclei	31.0	7.3		
Two nuclei	11 5	87.4	26.6	
One nucleus	0.7	4.3	73.4	100.0
	100.0	100.0	100.0	100.0

those with reduced and none with slight or no cytoplasm had undergone the second division. It is not at all probable that those in the last three groups would be able to function by the time the pollen was shed, even if they developed further.

TABLE VI

RELATION OF NUCLEAR DEVELOPMENT AND CYTOPLASMIC CONDITION IN POLLEN AT SHEDDING TIME

Material	Cytoplasm	Nuclei					Per cent
		Sperms	3	2	1	Empty	
dicoccum	Normal	1850	9	4	1		95.3
	Reduced	4	8	12	5		1.5
	Slight		3	20	12		1.8
	None					29	1.5
	Per cent	94 8	1.0	1.8	0.9	1 5	
F_1 vulgare × dicoccum	Normal	925	63	45			76 1
	Reduced	59	117	6			13 5
	Slight		5	21	23		3 6
	None					92	6 8
	Per cent	72 7	13 6	5.3	1.7	6 8	
F_1 vulgare × durum	Normal	817	92	97	1		64.5
	Reduced	40	62	187	36		20.7
	Slight		26	61	72		10.2
	None					71	4.6
	Per cent	54.9	11.5	22.1	6.9	4.6	
F_1 vulgare × persicum	Normal	1652	88	63			73.3
	Reduced	62	145	113	31		14.2
	Slight		19	79	82		7.3
	None					126	5.1
	Per cent	69 6	10.2	10.4	4.6	5.1	.

Finally, the conditions in pollen of mature stamens which were about to dehisce are shown in Table VI. From 27 to 45% of the F_1 pollen had not yet organized sperms and would be unable to function. As at earlier stages, most of those which were retarded in nuclear development showed some degree of reduction in cytoplasmic contents, but a considerable proportion which appeared normal with respect to the cytoplasm were still without properly organized sperms even at this late stage.

Several investigators have reported that the percentage of bad pollen in these hybrids is from 10 to 15. From the data in Table VI it appears that in those papers only that pollen was reported as bad which we have classified as having little or no cytoplasm. The previous accounts apparently did not distinguish from the normal, the 15 to 20% with a "reduced" amount of cytoplasm. Most of these "reduced" grains have no properly organized sperms and many have not even undergone the sperm division at the time the pollen is shed. It is very doubtful whether they would be ready to germinate and certain that they would not compete successfully with the normal grains which possess sperms. To the frequently reported 10 or 15% obviously

sterile pollen should therefore be added the 15 or 20% with reduced cytoplasm and retarded nuclear development. In addition about 10% of the grains with apparently normal cytoplasm (6 or 7% of all grains) are also retarded in nuclear development.

It was shown in an earlier section that the grains in which the nuclear development is retarded have chromosome numbers intermediate between those of the parents. This is one cause of the failure to function on the part of many grains with intermediate numbers. The chromosome combination may be so unfavorable as to cause the early abortion of some grains, or it may only retard their development so that they are not ready to function at the time the pollen is shed.

Germination of Pollen

In the previous section it was shown that in addition to the obviously sterile pollen grains there are many in which the nuclear development is so retarded that they would probably not be ready to germinate when the anthers dehisce. Experiments were therefore carried on to test the germination of F_1 pollen in comparison with that of the parents. Watkins (4) experimented on *vulgare* × *turgidum* pollen and extracts from his report are as follows: "With regard to the actual proportion of pollen that germinates it is difficult to reach a definite conclusion.... Allowing a proportion of 0.2 for aborted grains and assuming, in accordance with the indications of Table 8 that about 80 per cent of the pollen that was able to germinate had done so, we may put the proportion of F_1 pollen that can germinate on F_1 stigmas at about 0.10. Similarly on Rivet (*turgidum*) stigmas it appears that germination may be as high as about 30 per cent." On *vulgare* (Lion) it was only 5%.

Methods

Numerous unsuccessful attempts were made to germinate wheat pollen in various solutions at different concentrations. The results were no better with agar and sugar. Some success was attained by dusting the dry pollen on a coverglass and inverting it over a moist cell, but the results were not consistent and no way could be found of controlling conditions so as to obtain consistent results.

The authors then adopted the method of examining stigmas which had been pollinated previously. This had the advantage that conditions were more nearly natural, but also the disadvantage that some ungerminated grains might be lost in handling. Special experiments designed to test the latter point showed, however, that the loss was negligible when proper methods of handling were used.

Many preliminary experiments were carried on in order to test fixatives, stains and methods of handling, to determine when the stigmas were most receptive and how long it took the grains to germinate. The procedure finally adopted was as follows:

The spikes which were to furnish the stigmas were emasculated and protected when just clear of the sheath, 20 flowers from the 10 central spikelets being

used and the rest removed. Three to five days later when the stigmas were fully receptive, as judged by their well-feathered condition and by the separation of lemma and palea, they were artificially pollinated. To ensure that the pollen was fully mature and all types of grains properly represented, spikes were chosen in which some anthers had already dehisced. The tops were clipped from neighboring spikelets and the ripe stamens which emerged immediately were used individually. The spikes were then protected.

Ten to twelve hours later the stigmas were removed and all from one spike mounted on a slide. In order to reduce handling to a minimum they were mounted in a medium which acted both as fixative and stain, the most successful being lactic phenol colored with methylene blue. The best four to six stigmas on a slide were selected, the choice depending on a favorable number and distribution of grains and on relatively good germination (this indicating good receptivity of the stigma).

Owing to the much branched, feathery nature of the wheat stigma it was impossible to make a satisfactory study of the relative rates of pollen tube growth.

Results

In spite of the greatest care, different preparations of the same kind of material show considerable variation in the percentage of germination. The greatest variation among the stigmas of one F_1 plant pollinated by F_1 pollen is from 8.3 to 23.1%. And among all the 36 stigmas of this type on which full counts were made the variation is from 6.8 to 27.4. These variations apparently depend on varying degrees of receptivity of the stigmas and of moisture content of the flowers. In view of the number of grains examined and the consistency of the differences between F_1 and parental pollen, the authors believe the results give an approximately correct picture of the situation.

TABLE VII

Germination of pollen

Pollen	Stigmas	Per cent ungerminated grains of different cytoplasmic types						Germinated	Number of grains
		Normal	Reduced	Slight	None	Burst	Total		
vulgare 1	vulgare 1	5.9	3.4	1.7	5.9	6.3	23.3	76.7	236
vulgare 2	vulgare 2	4.7	2.9		3.2	1.5	12.3	87.7	339
dicoccum	dicoccum	14.6	1.9	1.3	3.9	8.1	29.9	70.1	308
persicum	persicum	9.8	3.3	1.3	9.8	2.6	26.8	73.2	153
vulgare 2	F_1	4.3	2.9	1.1	2.5	0.9	11.7	88.3	310
F_1	dicoccum	26.6	16.9	14.1	7.6	13.3	77.2	22.8	424
F_1 (dicoccum) (1)	F_1 (dicoccum)	37.9	28.1	10.7	4.6	7.4	88.7	11.3	327
F_1 (durum)	F_1 (durum)	31.6	21.4	17.3	13.8	4.6	88.8	11.2	916
F_1 (persicum)	F_1 (persicum)	41.2	15.5	12.0	7.3	11.2	87.4	12.6	658
F_1 (average)	In stamen	71.5	15.9	7.2	5.4				4378

(1) *All hybrids are between vulgare and the species mentioned.*

The data are summarized in Table VII. The grains recorded as germinated include those in which only the tip of the tube had protruded. The ratio of such grains to those with well-developed tubes is about 1 to 6. It is of course impossible to classify the germinated grains according to their cytoplasmic contents as is done in the case of the ungerminated grains because the cytoplasm of the former moves into the tube. The bursting of a small percentage of the grains apparently occurs in the flowers before the removal of the stigmas, although a small amount of it may be due to the handling.

The table shows that, under the conditions described, 70 to 88% of parental pollen grains germinate, but that only 11 or 12% of the pollen of three kinds of F_1 germinate on F_1 stigmas. These percentages are in agreement with the general conclusions reached by Watkins.

The ungerminated grains with less than the normal amount of cytoplasm constitute a somewhat higher percentage of all grains than is the case when the pollen is shed. This shows that none of the grains which are deficient in cytoplasm are able to germinate, even though the deficiency is slight. In addition there must be some influence which raises the proportion of cytoplasm-deficient grains above that found at pollination time. No evidence could be found that this was due to the falling-off of grains with normal cytoplasm. It may be due to swelling of grains on the stigma or to errors in classification. Some of the grains which had germinated and given up part or all of their cytoplasm to the tube may have become detached from the tube in handling, and recorded as ungerminated and deficient in cytoplasm. In some cases also the position of the germination pore may have been such as to make detection of the tube difficult. The percentage of germination in all cases may therefore have been somewhat higher than recorded. But even the maximum allowance for these causes would leave the germination of F_1 pollen less than 25%.

Besides those with deficient cytoplasm the ungerminated grains include a surprisingly high percentage of those with apparently normal cytoplasm. About 70% of mature pollen grains, still in the stamens, are normal in their cytoplasmic content; about 35% of all grains that have been in contact with stigmas are normal in cytoplasm and ungerminated. Consequently only about 50% of apparently normal grains germinate. It has been shown in a previous section, however, that about 10% of such grains with normal cytoplasm had not formed their sperms by shedding time. After allowance is made for these a high percentage, apparently normal in both cytoplasm and nuclei fail to germinate.

An examination of Table VII shows that the F_1 pollen germinates better on parental than on F_1 stigmas (22:11%). The difference is large enough to be significant. It cannot be attributed to unfavorable physical conditions in F_1 flowers, because parental pollen germinates equally well on F_1 and parental stigmas, as may be seen in the table. It appears, therefore, that the mutual relationship between F_1 pollen and F_1 stigmas is less favorable than that between the same pollen and parental stigmas.

References

1. SAX, K. Z. Abstamm. Vererb. Supp. 2: 1267-1284. 1928.
2. THOMPSON, W. P. and CAMERON, D. R. Genetics, 13: 456-469. 1928.
3. THOMPSON, W. P. and HOLLINGSHEAD, L. J. Genetics, 17: 283-307. 1927.
4. WATKINS, A. E. J. Genetics, 14: 129-171. 1924.
5. WATKINS, A. E. J. Genetics, 15: 323-366. 1925.

INHERITANCE OF RESISTANCE TO FOWL PARALYSIS (*NEUROLYMPHOMATOSIS GALLINARUM*)

II. ON A SIGNIFICANT DIFFERENCE IN THE INCIDENCE OF FOWL PARALYSIS IN TWO GROUPS OF CHICKS[1]

By Jacob Biely[2], Elvira Palmer[3] and V. S. Asmundson[4]

Abstract

Data are presented on two groups of thirty chicks each, hatched from a susceptible and an apparently resistant flock. There was a significant difference in the incidence of fowl paralysis and lymphomatous tumors in these two groups. This is interpreted to mean that there is an inherent difference in susceptibility and resistance to fowl paralysis and lymphomatous tumors.

Comparatively little nformation is as yet available regarding the mode of transmission of fowl paralysis (*Neurolymphomatosis gallinarum*). Pappenheimer, Dunn and Seidlin (8) are the only investigators who have reported success in transmitting fowl paralysis by artificial inoculation of newly hatched chicks. They report that typical lesions were found in approximately 25% of the inoculated chicks, while in control chicks kept under laboratory conditions the incidence of the disease was about 7%. These investigators have also observed that some of the experimentally inoculated chicks developed lymphomatous tumors. It may be noted that Mathews and Walkey (5) are of the opinion that there is no connection between paralysis and the common lymphadenomas of the fowl. They further state that these are inherited as a simple Mendelian recessive.

Doyle (3) reported that several birds hatched from eggs laid by an affected flock developed typical paralysis and iritis. The first case of paralysis occurred at 47 days of age. Control birds kept under observation until ten months of age did not show evidence of neuritis or paralysis. Doyle states, "It appears more than probable, then, that paralysis may be transmitted from parent to offspring in much the same way as bacillary white diarrhoea."

Marginson and McGaughey (4) report that the histories of 17 outbreaks of fowl paralysis afford strong evidence that the disease may be transmitted through the egg and by young chicks. Beaudette and Hudson (2) state that "while there is much to be done before the question of transmission can be finally settled, the evidence at hand (based on field observations) is strongly in favor of the conception of transmission through the egg". These authors are also of the opinion that the appearance of paralysis on a hitherto non-infected farm is always preceded by the introduction of hatching eggs, baby chicks, or breeding stock from an affected flock.

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The present report deals with one phase of the authors' investigations conducted during the past year, namely, the incidence of fowl paralysis and lymphomatous tumors in chicks hatched from (1) a paralysis affected flock, and (2) a flock apparently free from paralysis.

Material

The breeding flock used in this investigation consisted of 119 Rhode Island Red pullets mated to 6 Black Orpington males. Data presented in a previous report (Asmundson and Biely (1)) dealing with the inheritance of resistance to fowl paralysis, showed that 49 of these 119 pullets belonged to families free of paralysis and lymphomatous tumors. Seventy pullets belonged to families in which paralysis or lymphomatous tumors were known to be present. Of these seventy, 51 appeared to be normal, 13 became paralyzed, 2 had lymphomatous tumors only, and 4 showed paralysis and lymphomatous tumors.

Of the six males, two were from apparently resistant families. The other four were from two susceptible families. In the latter group out of thirteen sisters in one family, one became paralyzed, while out of seven sisters in the second family one became paralyzed and one died from lymphomatous tumors.

Eggs from the flock of 119 Rhode Island Red pullets were collected in November and December. At that time several birds died from paralysis and lymphomatous tumors. Some of the birds which laid during the period when the eggs were collected for hatching subsequently developed paralysis or died from tumors. Undoubtedly, therefore, some of the eggs laid by these pullets were incubated with the eggs used in this experiment, although pedigrees are not available to prove this.

The chicks from the above mating hatched January 1, 1931, and were transferred to wire battery brooders placed in a separate room, in a building where a few normal and paralyzed birds were kept in cohabitation.

The first case of paralysis in these chicks developed at the end of exactly eight weeks, or 56 days, while the birds were still in the battery brooder. Since the birds grew rapidly and were too large for the battery brooders, some were disposed of, while 30 well-grown chicks were placed in a separate house and kept on a wire floor.

Experimental

Group 1

On March 20, when the birds were 79 days old, one of the 30 birds developed paralysis of the left leg, and on post-mortem examination showed gross lesions in the brachial plexus, the spinal cord, the lumbo-sacral plexus, sciatic nerves, and a lymphomatous tumor of the ovary (Fig. 5). Considering the age of this bird, the gross lesions were very marked and extensive. Photomicrographs showing the massive lymphoid infiltration of the nervous system of this bird are presented in Figs. 1, 2, 3 and 4, and of the ovary in Fig. 6. These figures illustrate the typical conditions observed in affected birds.

The clinical symptoms and gross lesions of the 30 birds are shown in detail in Table I, and summarized in Table II. Thirteen females and nine males were examined before May 15. Of the 13 females 7 became paralyzed and showed gross lesions in the nervous system, and four of these also showed lymphomatous tumors of the ovary. Two of the remaining six birds showed lymphomatous tumors of the ovary, but did not show any gross lesions in the nervous system.

TABLE I

INCIDENCE OF PARALYSIS AND LYMPHOMATOUS TUMORS IN CHICKS HATCHED FROM A FLOCK AFFECTED WITH PARALYSIS

Sex[1]	Number of bird	Description	Age in days	Gross lesions in the nervous system[2]	Lymphomatous tumors[3]
F	715	Paralyzed	79	X	X
F	676	Died	81	—	X
F	723	Paralyzed	89	X	X
F	607	Died	92	—	—
F	662	Paralyzed	96	X	X
F	633	Paralyzed	97	X	—
F	689	Paralyzed	98	X	X
M	687	Killed	100	?[4]	—
M	612	Paralyzed	103	X	—
M	620	Paralyzed	107	X	—
M	660	Killed	107	—	—
M	711	Killed	110	—	—
M	688	Killed	110	?	—
M	682	Killed	110	?	—
M	622	Killed	110	?	—
F	692	Paralyzed	119	X	—
M	699	Killed	125	—	—
F	695	Killed	126	—	—
F	679	Killed	126	—	—
F	657	Paralyzed	126	X	—
F	611	Killed	132	—	—
F	685	Killed	132	—	X
F	675[5]	Died	200	—	—
F	678	Killed	215	—	—
F	680	Died	240	—	X
F	700	Killed	250	—	—
F	655	Killed	286	—	X
F	702	Killed	328	—	X
F	677	Killed	336	—	—
F	770	Killed	353	—	X

[1] *F = female; M = male.* [2] *X = lesions visible to the naked eye.* [3] *X = tumors visible to the naked eye.* [4] *? = lesions confined to the brachial plexus.* [5] *The last eight birds in this table are not strictly comparable to Group 2 in Tables III and IV since they were kept for more than 145 days.*

TABLE II

SUMMARY OF TABLE I

Number of birds		Paralysis only	Paralysis and tumors	Tumors only	No tumors or paralysis
Females	21	3	4	6	8
Males	9	2	0	0	7
Total	30	5	4	6	15

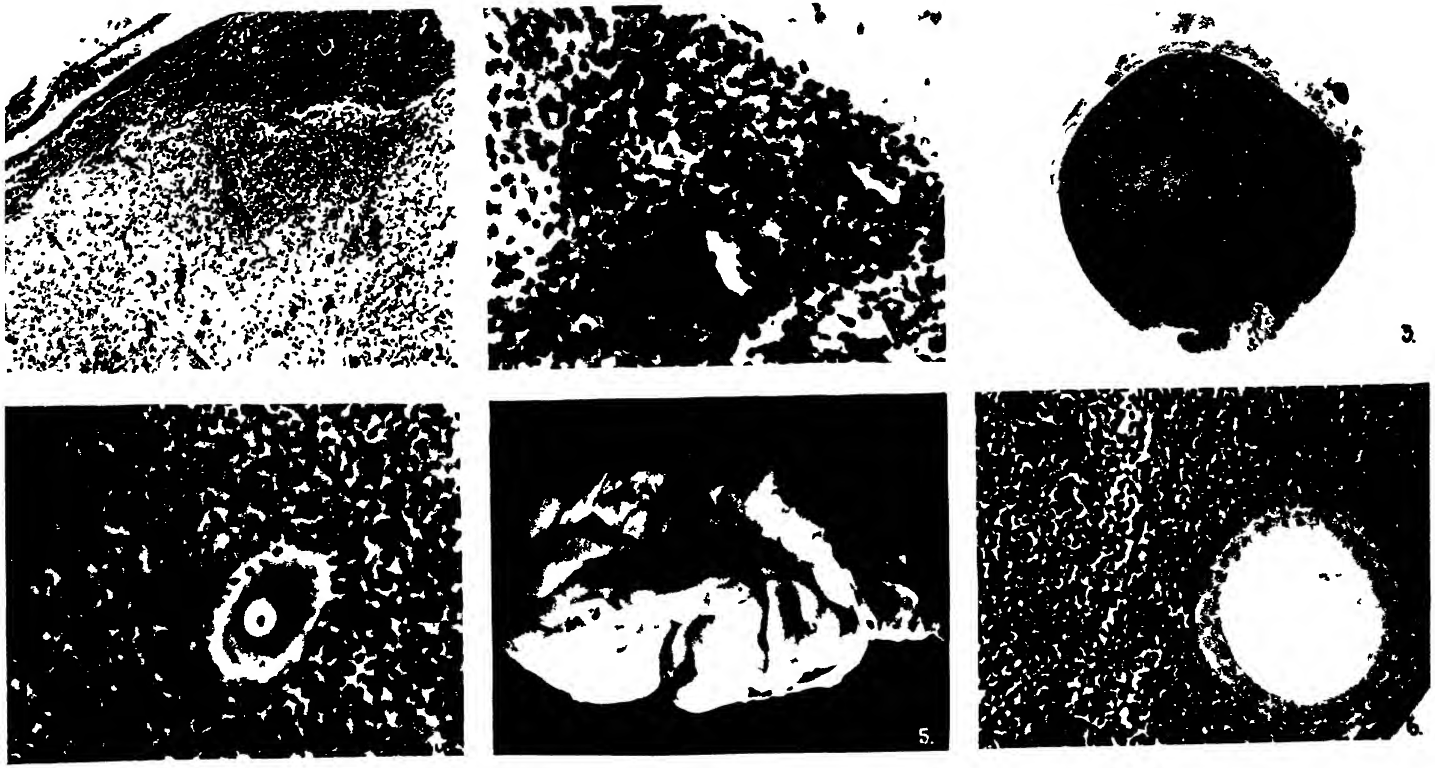

FIG. 1. Bird No. 715. Cross section of spinal cord showing massive perivascular and interstitial lymphoid infiltration. FIG. 2. Bird No. 715. Dense perivascular lymphoid infiltration in spinal cord, high power. FIG. 3. Bird No. 715. Brachial nerve root. Note almost complete replacement of nerve tissue by lymphoid cells. FIG. 4. Bird No. 715. Higher magnification of a part of the section shown in Fig. 3. Note the density of the infiltration and the uniform distribution of the lymphoid cells. FIG. 5. Bird No. 715. Lymphomatous tumor of the ovary. FIG. 6. Bird No. 715. Cross section of lymphomatous tumor of the ovary.

Two of the nine males were paralyzed and showed gross lesions in the nervous system. The remaining seven males were apparently normal, but on post-mortem examination four of these showed gross lesions in the brachial plexus. Had these seven birds been kept for a longer period they would possibly have developed paralysis.

The remaining eight pullets from this group of thirty began to lay when five months of age and were placed in a newly built house on clean ground. Two birds died from intercurrent causes; in one of these the ovary presented a lymphomatous condition. Two were killed because they were crop-bound. The remaining four showed definite signs of emaciation, diarrhea, and on post-mortem examination, characteristic lymphomatous tumors (about 3 to 4 inches in diameter) were found in the ovaries of three birds and generalized lymphomatous tumors in the fourth bird.

It is interesting to note that four Single Comb White Leghorn pullets, of unknown ancestry, and a male bird kept in the same pen with these eight pullets from May 25 to date (December 19), appear to be in excellent condition.

Group 2 (Control Chicks)

S.C.W. Leghorn chicks secured from a flock in which paralysis had not occurred *in previous years* served as controls. It should be noted, however, that out of approximately 2000 chicks hatched on this farm, four developed paralysis late in the spring and six adult birds developed paralysis in the autumn and winter. Furthermore, 8 to 9% of the chicks sold from this

TABLE III

INCIDENCE OF FOWL PARALYSIS IN INOCULATED CHICKS

No. of chick	Route and order of inoculation				Killed or died (age in days)	Lesions in nervous system[1]
	Leg cc.	Leg cc.	Breast cc.	Brain and leg cc.		
2488	0.5	0.5	0.5	0.2	60	—
74	0.5	0.5	0.5	0.2	84	—
157	0.5	0.5	0.5	0.2	96	—
55	0.5	0.5	0.5	0.2	102	—
91	0.5	0.5	0.5	0.2	102	—
81	0.5	0.5	0.5	0.2	104	—
96	0.5	0.5	0.5	0.2	104	—
87	0.5	0.5	0.5	0.2	124	—
84	0.5	0.5	0.5	0.2	124	—
		Brain	*Breast*	*Leg*		
2486		0.2	0.5	0.5	56	—
86		0.2	0.5	0.5	68	—
187		0.2	0.5	0.5	75[2]	X
27		0.2	0.5	0 5	103	—
2476		0.2	0.5	0 5	104	—
89		0 2	0.5	0.5	124	—

[1]— = *no lesions visible to the naked eye; X = lesions visible to the naked eye.*
[2] = *paralysis at 75 days.*
NOTE:—*The inoculations were performed when the chicks were 7-14 days old.*

flock to a farm where paralysis was prevalent for the past three years developed paralysis. No losses have been reported by others who purchased chicks from this farm.

The chicks secured as controls were raised in a wire battery brooder placed in a room opposite to the one in which the crossbred chicks (susceptible strain) were raised. One group of chicks (Lot A) was inoculated and kept in the upper deck of the battery brooder, while the other group (Lot B), which was not inoculated, was kept in a lower deck.

The chicks of Lot A were inoculated four times within a week, twice in the leg, once in the breast, and once n the brain and leg. The inoculum, which consisted of heavy suspensions of nervous tissue obtained from paralyzed birds, was freshly prepared prior to each series of inoculations. Table III shows the route of inoculation and the age at which the chicks died or were killed. It will be seen that one chick, No. 187, developed paralysis at 75 days, with gross lesions in the nervous system. The remainder did not show paralysis or gross lesions on post-mortem examination.

TABLE IV
INCIDENCE OF PARALYSIS AND LYMPHOMATOUS TUMORS IN UNINOCULATED CHICKS

Number of chicks	Killed or died (age in days)	Gross lesions in nervous system	Lymphomatous tumors[1]
1	60	—	—
1	96	—	X
1	102	—	—
2	104	—	—
3	128	—	—
3	134	—	—
4	145	—	—

[1]— = *no lesions visible to the naked eye; X = lymphomatous tumor of the ovary present.*
NOTE:—*There was no paralysis in this group of chicks.*

Table IV shows the age at which the uninoculated chicks (Lot B) died or were killed. None of the chicks developed paralysis or showed gross lesions in the nervous system. One chick, No. 2479, showed lymphomatous tumors, without paralysis, at 96 days. The remainder appeared normal on post-mortem examination.

Discussion

The data on the two groups of chicks, which were based on clinical symptoms and careful post-mortem examinations, are presented in Tables I, III and IV. Only chicks that died or were killed at certain intervals, after 56 days, were examined. The majority of the chicks were over two and one-half months (75 days) of age, when examined. The observations, except in the case of eight pullets (Table I) were concluded before the chicks were 145 days old. While this period of observation may not have been long enough to permit paralysis to develop in all cases, since the disease may occur up to 15 months of age, it undoubtedly includes the large majority of cases.

It is apparent from a study of the data in Tables I to IV that there was considerable difference in the incidence of paralysis and tumors in the two

groups of chicks. Thus 11 out of 30 chicks (Group 1), or 37%, hatched from a susceptible flock, showed paralysis, paralysis with tumors, or tumors only; whereas in Group 2, 1 of the 15 inoculated chicks developed paralysis, and 1 of the 15 uninoculated chicks developed a lymphomatous tumor. Thus only 2 out of the 30 chicks, or 6.6%, in Group 2 showed fowl paralysis or lymphomatous tumors, as contrasted with 11* out of the 30 chicks hatched from the susceptible flock. The difference in the incidence of paralysis and tumors in these two groups appears to be significant. This agrees with data presented in a previous paper (Asmundson and Biely, (1)) which indicated that resistance or susceptibility to fowl paralysis is inherited.

It is evident that neither the severe inoculation, repeated four times, with heavy suspensions of nervous tissue from paralyzed birds, nor the fact that the chicks were housed in a building in which paralyzed birds were kept, appeared to influence the resistance of the majority of the chicks to paralysis. In any large population there are likely to be susceptible individuals, and it is not improbable that the two birds (inoculated and uninoculated) that developed paralysis or lymphomatous tumors, were susceptible to fowl paralysis. The possibility of the presence of a small proportion of susceptible chicks among the controls is further indicated by the fact already mentioned that about 8% of the chicks, raised on a farm where paralysis was endemic, developed the disease.

It is of considerable importance to determine whether, as suggested by Doyle, paralysis can be transmitted from the parent stock to the progeny through the egg. Our data do not permit definite conclusions on this point. Nevertheless the early appearance of paralysis and gross lesions in the nervous system of some of the chicks points to transmission through the egg.

It is interesting to note, in connection with the chicks hatched from the susceptible stock, that the parent stock did not develop paralysis until they were from 5 to 10 months old, while the progeny developed paralysis and gross lymphomatous lesions at a comparatively early age, or less than three months. Both paralysis and lymphomatous tumors occurred at about the same age, possibly indicating that the incubation period of the two conditions is about the same (2 to 10 months). The relation of the tumors to paralysis will be discussed in a subsequent paper.

While the relation of fowl paralysis and lymphomatous tumors cannot be determined on the basis of the data presented in this and a previous paper (1) it is of interest to note that in the groups so far studied both tend to occur together more frequently than would be expected on the basis of chance. Finally, the data presented in this paper also furnish additional evidence in favor of the view that resistance and susceptibility to fowl paralysis and lymphomatous tumors are inherited.

**Four cases of lymphomatous tumor occurred in the eight birds which were kept after 145 days of observation, making a total of 15 affected birds (see Table I).*

Summary

1. The data reported in this paper were based on two groups of chicks observed for periods ranging from 60 to 145 days. Group 1 was hatched from a susceptible flock, while Group 2 was hatched from an apparently resistant flock.

2. Out of 30 chicks from Group 1, 5 developed paralysis only, 4 paralysis with lymphomatous tumors, and 2 lymphomatous tumors only, making a total of 11 affected chicks. Four apparently normal males showed gross lesions in the brachial plexus.

3. Out of 15 inoculated chicks of Group 2, 1 developed paralysis; and out of 15 uninoculated chicks of Group 2, 1 developed lymphomatous tumors only.

4. The early incidence of fowl paralysis points to transmission through the egg.

5. The first case of paralysis and lymphomatous tumor in the same chick was observed at 79 days, indicating that the period of "incubation" is similar.

6. There was a difference in the incidence of fowl paralysis and lymphomatous tumors in the two groups of chicks. This is interpreted to mean that there is an inherent difference in susceptibility and resistance to fowl paralysis and lymphomatous tumors.

Acknowledgment

The authors wish to acknowledge their gratitude to Dr. H. W. Hill, Head of the Department of Bacteriology, and Professor E. A. Lloyd, Head of the Department of Poultry Husbandry, for their invaluable advice and unfailing interest in the investigations of fowl paralysis.

References

1. Asmundson, V. S. and Biely, J. Can. J. Research, 6: 171-176. 1932.
2. Beaudette, F. R. and Hudson, C. B. New Jersey Agr. Exp. Sta. 19: 1-4. 1931.
3. Doyle, L. P. Poultry Sci. 8: 159-160. 1929.
4. Marginson, G. C. and McGaughey, C. A. Vet. Record, 18: 573. 1931.
5. Mathews, F. P. and Walkey, F. L. J. Cancer Research, 13: 383-400. 1929.
6. Pappenheimer, A. M., Dunn, L. C. and Cone, V. Storrs Agr. Exp. Sta. Bull. 143: 187-290. 1926.
7. Pappenheimer, A. M., Dunn, L. C. and Cone, V. J. Exptl. Med. 49: 63-86. 1929.
8. Pappenheimer, A. M., Dunn, L. C. and Seidlin, S. M. J. Exptl. Med. 49: 87-102. 1929.

COMPARISON OF EFFICIENCY OF THE RAPID WHOLE BLOOD AGGLUTINATION TEST WITH THE SERUM AGGLUTINATION TEST FOR PULLORUM DISEASE[1]

By Jacob Biely[2] and William Roach[3]

Abstract

The results obtained with the rapid whole blood agglutination test for pullorum disease, applied in the field, agree closely with the results secured with the rapid serum agglutination test, applied in the laboratory.

The accuracy of the diagnosis was found to depend upon the training and experience of the technician. When the whole blood agglutination test was applied by inexperienced persons, the results obtained differed from the laboratory test by 12% as compared with a difference of 1.3% when the whole blood agglutination test was applied by an experienced technician.

The rapid whole blood agglutination test was found to lend itself very readily to practical application in the field. The extremely low cost makes feasible the application and repetition of the test on a large scale.

Since it is known from previous work that one agglutination test will not eliminate all carriers of pullorum disease, the rapid whole blood agglutination test should be applied several times a year until at least two successive negative tests are obtained on each bird of the flock.

The recently developed "rapid whole blood agglutination test" for the detection of pullorum disease has given variable results, when compared with those of the serum agglutination test. It therefore appeared to be worthwhile to undertake further work for the purpose of comparing the results of the rapid whole blood agglutination test for pullorum disease in the field with the results of the rapid serum agglutination test in the laboratory. The present series of experiments were designed to test also the influence of the personal factor on the diagnosis of pullorum disease by the rapid whole blood agglutination test in the field. The need for further work along this line is indicated by the literature, which is here briefly reviewed.

In 1929 Bunyea, Hall and Dorset (7) reported that they had developed a simplified agglutination test in which whole blood and a specially prepared antigen were used. Bunyea and Hall (6) made a comparative study of the rapid whole blood agglutination test and the tube agglutination test, and reported 87.6% agreement on six flocks. Eight per cent of the cases reacted to the simplified test but not to the tube test; 5% reacted to the tube test but not to the simplified test.

J. R. Beach and Michael (1), Brandly (4), Sawyer and Hamilton (11), and Broerman (5), did not consider the simplified test to be a satisfactory substitute for the serum agglutination test. Bleecker (3) concluded that in the hands of an experienced operator the whole blood agglutination test is fully as efficient as the tube agglutination test.

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The whole blood agglutination test has met with the marked approval of German investigators, and was found on the whole to be as accurate as the serum agglutination test (9, 10, 13, 14).

Schaffer, Macdonald, Hall and Bunyea (12) developed an improved antigen for the whole blood agglutination test, which had advantages over the old one in that it was adequately preserved, and was deeply stained by means of crystal violet.

Coburn and Stafseth (8) improved the antigen for the whole blood agglutination test (by adding 0.5% phenol and gentian violet) and used standardized dropper pipettes for measuring antigen and blood.

Method of Conducting the Tests

The details of the technique of the rapid serum agglutination test and the interpretation of the results have been reported in a previous paper (2). For purposes of the present paper, it is sufficient to state that the antigen (four strains of *S. pullorum*) is adjusted to a turbidity fifty times greater than tube 0.75 of McFarland's nephelometer; 0.4 cc. of serum and 0.2 cc. of standardized antigen are used; readings are made after five minutes incubation at 37° C., with second readings after two to three minutes at room temperature.

The technique employed with the rapid whole blood method varied in certain details from that generally recommended. The use of a flat glass plate was found unsatisfactory on account of the rapid drying of the blood smears, the deposition of dust, and a certain awkwardness in mixing the blood and the antigen. Instead, two white porcelain spot plates, with twelve sunken wells, were used. These were kept covered with plain glass, to exclude dust. Each plate had a manufacturer's trade mark on one edge, which was kept for reference as a starting point.

In each well, a drop of antigen was placed with a dropper designed to deliver 0.05 cc. As the birds were bled, a drop of blood was taken direct from the wing with an inoculating needle, the loop of which, in accordance with the recommendations of Schaffer *et al* (12), was $\frac{3}{8}$ in. in diameter. The blood and the antigen were mixed with the inoculator, and the plate was rotated to ensure complete mixing. The loop was rinsed in dilute coal tar disinfectant and dried after each test. The plates were washed in a similar solution which was kept hot, then dried with a clean towel.

By the use of two spot plates it was possible to read each test as the third succeeding test was made. A watch was used to time the reactions. Usually two or three readings were made. The diagnoses were recorded as positive, suspicious or negative. Positive and suspicious reactors were removed from the flocks.

Leg band numbers were recorded in four rows of three numbers each, to conform to the position of the wells of the spot plates, the trade mark being kept on the left of the operator when mixing blood and antigen.

Two poultry crates were used, the 12 birds tested on one plate being placed in one crate, the birds being released and the reactors removed as the readings of each plate were completed. Thus only two sets of bands, of different colors, numbered from one to twelve, were required.

The blood was secured by pricking the ulnar vein with a sharp Bard Parker blade No. 11 at the point at which the vein passes over the juncture of the humerus and the radius-ulna. Usually, very little or practically no bleeding followed when the blood was taken at this point, whereas a considerable loss of blood often occurs if an incision is made in the ulnar vein nearer to the body, as in the case of collecting blood for the serum agglutination test. With the above technique, from 100 to 125 birds were tested in one hour.

Blood samples from each bird were sent to the laboratory for the rapid serum agglutination test, this blood being collected in the usual manner.

Antigens for the rapid whole blood method were secured from three sources: (1) through the courtesy of Dr. M. Dorset, Bureau of Animal Industry, U.S. Department of Agriculture, Washington, D.C.; (2) and (3), from two commercial firms.

Results

Some preliminary tests were made in the laboratory with the B.A.I. antigen before any work was done in the field. In order to secure some idea of the diagnostic value and the sensitivity of the stained antigen used for the rapid whole blood method, several hundred blood samples were tested in the laboratory by the usual rapid serum agglutination test, and the sera retested in the same manner with the stained antigen. At first, difficulties were experienced

TABLE I

Comparative whole blood and rapid serum agglutination tests

Flock	Number of birds	Whole blood antigen			Rapid serum antigen			Total disagreement
		Positive	Suspicious	Negative	Positive	Suspicious	Negative	
1	91			91			91	0
2	55			55			55	0
3	78			78			78	0
4	100			100			100	0
5	127	5		122	5		122	0
6	118	2		116			118	2
7	100			100			100	0
8	288	19		269			288	19
8 a	198	5		193			198	5
9	74	14		60			74	14
10	40	6	1	33	7	1	32	3
11	219	39		180	42	3	174	11
12	71	10	3	58	9	1	61	3
13	188	10		178	13		165	3
14	137			137			137	0
15	40			40			40	0
16	322			322			322	0
17	62	20		42	20	1	41	3
12 a	168	50	2	116	52	1	115	4
13 a	224	13		211	13		211	0
18	29			29			29	0
19	93	8		85	7		86	1
20	110	68		42	69		41	2
	2932	269	6	2657	237	7	2678	. 70

in interpreting the reactions secured with the stained antigen. However, after gaining some experience in reading the tests with the stained antigen, practically identical results were secured.

The preliminary evidence of these tests indicated that the rapid whole blood stained antigen was as sensitive as the rapid serum antigen used in this laboratory, and that experience is necessary for the proper interpretation of the reactions.

Table I shows the comparative results secured with the two methods from twenty flocks, involving 2932 birds. It will be seen that, with the exception of three flocks (8, 8a and 9), the rapid whole blood agglutination test agreed very closely with the rapid serum agglutination test. The last column in the table shows the total number of birds that gave a positive or suspicious reaction with the serum agglutination test and a negative reaction with the whole blood method, and *vice versa*. Seventy birds, or 2.3% out of 2932 tested, failed to agree. If we exclude the three flocks (8, 8a and 9) to which reference will be made later, only 28 out of 2932 tests, or 1.3%, failed to agree. It will be further seen that at the close of the investigation, because of the experience gained, there developed practically complete agreement in the diagnoses by the whole blood and rapid serum agglutination tests. Beginning with flocks Nos. 8, 8a and 9, a new antigen was used. Discrepancies occurred in the first three flocks tested with the substituted antigen, which was decidedly more sensitive and rapid than the first one used. After a certain amount of experience with the second antigen, a close agreement with laboratory diagnoses was secured, and this close agreement was maintained when a third antigen was introduced. The first and third antigens used in the field were those secured from commercial firms.

On the basis of the data presented in Table I, it may be concluded that the rapid whole blood agglutination test and the rapid serum agglutination test are of equal value in the diagnosis of pullorum disease.

Influence of the Personal Factor

In order to test the influence of the personal factor in the interpretation of the field results, antigen was sent to several persons interested in the rapid whole blood agglutination test, but without experience. Instructions in technique and interpretation of results were sent to each. When their field tests were completed, the corresponding blood sample was sent to the laboratory for comparative diagnosis.

The results of this comparative study are given in Table II. The data show that there was considerable disagreement between these field results and the laboratory results; in fact, from the diagnostic point of view these field results were worthless. The last column in Table II shows that 145, or 12%, out of 1201 tests failed to agree. This contrasts markedly with the practical agreement of the two tests when the field test was made by an experienced person.

One of the flocks included in Table II was later retested simultaneously by two fieldmen, and a very close agreement with the laboratory test was secured. This flock was No. 11 in Table I and No. 1 in Table II.

TABLE II

THE EFFECT OF THE PERSONAL FACTOR ON THE ACCURACY OF THE WHOLE BLOOD AGGLUTINATION TEST IN THE DIAGNOSIS OF PULLORUM DISEASE

Person	Flock	Number of birds	Whole blood antigen			Rapid serum antigen			Total dis-agree-ment
			Positive	Suspi-cious	Negative	Positive	Suspi-cious	Negative	
"A"	1	225		5	220	42		183	47
	2	112		4	108			112	4
	3	86		6	80			86	6
"B"	4	460	21	2	437			460	23
	5	193	28		165			193	28
	6	49	5		44			49	5
"C"	7	33	4	3	26	9	1	23	15
	8	30	14	3	13	3		27	16
"D"	9	13			13	1		12	1
		1201	72	23	1106	54	1	1145	145

Discussion

Data secured in this investigation show that 98.7% of agreement was secured in the diagnosis of pullorum disease by the rapid whole blood agglutination test and the rapid serum agglutination test. In order to get such close agreement it is necessary that the test be made by a trained and experienced person. It is interesting to note here that Bleecker (3) obtained 91.15% agreement with 2159 birds when tested by the whole blood and tube agglutination tests, most of the discrepancies having occurred with the first few flocks tested. At the close of the experiment Bleecker obtained almost identical results with the two tests.

In the case of two flocks involving 140 birds, the tube agglutination test was used in a dilution of 1 to 25. Seventy-one birds reacted positively with the whole blood antigen in the field, while 69 reacted positively with the tube agglutination test. It is apparent from this that the antigen for the whole blood agglutination test is very sensitive in detecting pullorum disease carriers.

The effects of lack of experience and training in interpreting the whole blood agglutination test are clearly demonstrated in Table II. It is obvious that in several of the flocks used in the investigation the errors might have resulted in the discarding of valuable breeders, while in others it might have resulted in leaving numerous diseased birds in breeding flocks.

The importance of experience in the interpretation of agglutination tests is further emphasized by a case in which, out of a flock of 700 to 800 birds, 140 were diagnosed as "positives" by the rapid serum agglutination test, applied by a commercial laboratory, while actually only 8 out of the 140 reacted positively when tested in two other laboratories by experienced technicians.

In the case of flocks 4 and 5, Table II, the reactors were re-bled and submitted to a commercial laboratory for retesting. In the case of flock 4 no positive reactors were reported, while in the case of flock 5, three birds were reported as suspicious. One of these was submitted for post-mortem examination; this bird did not show pullorum disease lesions.

Since the field test appears to be as accurate as the laboratory test in the diagnosis of pullorum disease, the advantages of the rapid whole blood agglutination test over the tube and agglutination serum tests are obvious. The rapid whole blood agglutination test lends itself very well to the control of pullorum disease, and therefore its use in the field is warranted.

The advantages may be stated briefly as follows: 1. There is no necessity to draw a quantity of blood from a bird, a single drop sufficing for the test. 2. Reactors can be removed from the flock as soon as the test is applied, which eliminates the necessity of going through the flock a second time in a search for the reactors, which is necessary with the other tests. 3. There is no need of a bird being permanently banded. 4. The test can be performed at any time of the year. 5. By means of repeated testing pullorum disease could be eradicated in one season. 6. No expensive equipment is required, hence the cost is relatively low.

Acknowledgment

The writers wish to thank Messrs. M. H. Ruttledge and T. J. Smith, President and Managing Director respectively, of the R.O.P. Poultry Breeders' Association of British Columbia, and Mr. W. J. Spence of the Canadian Hatcheries Limited, for their deep interest in this work and their ready assistance in providing flocks for making the double test. They wish to express also their appreciation to Dean F. M. Clement of the Faculty of Agriculture, University of British Columbia, for making a special grant to conduct this investigation, and to Dr. H. W. Hill for permission to use the facilities of the Department of Bacteriology.

References

1. Beach, J. R. and Michael, S. T. Univ. of California, Berk., Bull. 486: 1-31. 1931.
2. Biely, J. Can. J. Research, 5: 693-706. 1931.
3. Bleecker, W. L. J. Am. Vet. Med. Assocn. 78: 518-526. 1931.
4. Brandly, C. A. Vet. Med. 25: 154-159. 1930.
5. Broerman, A. Vet. Med. 25: 197-198. 1930.
6. Bunyea, H. and Hall, W. Proc. Fourth World's Poultry Congress (Lond.) Sec. C. 554-560. 1930.
7. Bunyea, H., Hall, W. J. and Dorset, M. J. Am. Vet. Med. Assocn. 75: 408-410. 1929.
8. Coburn, D. R. and Stafseth, H. J. J. Am. Vet. Med. Assocn. 79: 241-243. 1931.
9. Miessner, H. and Berge, R. Deut. Tierärztliche Wochenschrift. 401-406. 1930.
10. Peters, G. Intern. Rev. Poultry Sci. Abstract, 4:16. 1931.
11. Sawyer, C. E. and Hamilton, C. M. West. Wash. Expt., Sta. Puyallup, Wash. Bull. 17. 1930.
12. Schaffer, J. M., MacDonald, A. D., Hall, W. J. and Bunyea, H. J. Am. Vet. Med. Assocn. 79:236-240. 1931.
13. Schmid, G. Intern. Rev. Poultry Sci. Abstract, 4: 15. 1931.
14. Wagner, K. Arch. Geflugelkunde, 6: 244-256. 1930.

PHENOMENA OF PRIMENESS[1]

By Charles Kenneth Gunn[2]

Abstract

The condition of muskrat pelts known as "primeness" is found to be independent of dermal characteristics and to be a function of epidermal pigment distribution. The microscopic characteristics of prime and unprime fur pelts are discussed and correlated with the macroscopic characteristics. A method is suggested for detecting primeness in the skin of living animals.

Introduction

The characteristics of a pelt which determine its value as a "fur" are certain variable characters of the epidermis—the so-called pelage—and of the dermis—the so-called leather.

Two characteristics in particular are of primary commercial importance, those of thickness and primeness. Such factors as the color, uniformity, sheen, length and density of the pelage, are subsidiary to the characteristics of dermal thickness, and of epidermal and dermal primeness. Upon dermal thickness depends the success of the technical processes of dyeing and dressing, and the durability of the finished product. Upon primeness depends the color, sheen, length and density of the pelage and the pigmentation of the leather.

An exact knowledge as to what constitutes "primeness", which in the last analysis is the ultimate criterion of the pelt, is therefore of fundamental importance. The existing methods of estimating primeness are empirical, do not take into account the morphological and physiological factors which underlie this condition, nor can they be applied to living animals.

The aims of this investigation therefore were:—

(1) To ascertain the histological differences between prime and unprime pelts.

(2) To correlate seasonal changes in the pelt with the visible phenomena of moulting and the growth of new fur.

(3) To provide a method for the separation of prime from unprime pelts, based upon such histological differences that can be applied to the pelts of *living* fur-bearing animals.

Methods

The material consisted of living male muskrats (*Fiber zibethicus albus*) from Washow Bay, on the west coast of Lake Winnipeg, and a large number of treated and untreated pelts. Male animals were used to avoid any variations in the pelt which might result from secondary effects caused by oestrous periods. One rat pelted on September 26, showed marked unprimeness along the mid-dorsal region; from these unprime pigmented areas microscopic sections were made. Sections of a prime pelt were prepared from a muskrat, from the same ranch, pelted on February 1.

The microtechnical procedure consisted of Bouin fixation, followed by haematoxylin, eosin or picro-fuchsin stains.

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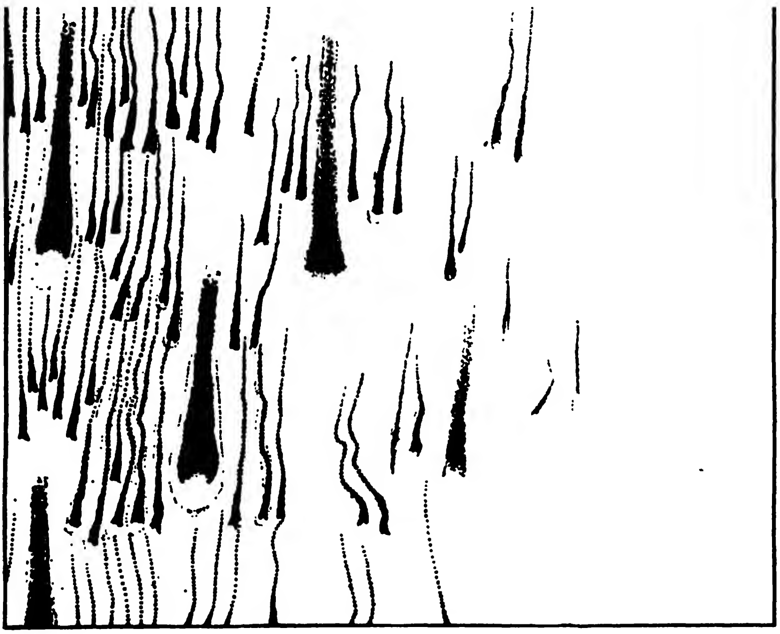
Contribution from the University of Manitoba.

[2] *Demonstrator in Zoology, University of Manitoba.*

Structure of the Pelt

By shearing the fur and carefully stripping the panniculus carnosus muscle from the untreated dried pelt, it is possible to examine and study the remaining portion of the leather directly with the low power of the microscope.

In such preparations the field is seen to be strewn with irregular groups and rows of densely pigmented hair roots, embedded in a flesh-colored connective tissue (Text–fig. 1). The hair roots are of two kinds. A large type, the guard-hair roots, are scattered sparsely over the field; their pigment is diffusely distributed in the bulbous portion of their roots: a much smaller, but more numerous type, the underfur hair roots, are arranged in groups, either around



TEXT-FIG. 1. *Diagram showing the arrangement of hair roots at the junction of prime and unprime areas of muskrat pelt.*

the larger roots of the guard-hairs or in clusters and rows without a central guard-hair, and with the melanin granules so densely concentrated in the ampulla of the root as to give the root the appearance of a black wedge-shaped structure.

From the outer end of this wedge small blocks of pigment pass from the adjoining portion of the root, at first closely packed together, but becoming more definitely separated from each other, as they pass into the portion of the hair root nearer to the mouth of the follicle. In the roots of the underfur hairs, the melanin is confined completely to the medulla, while in the case of the guard-hairs it also permeates the cortex and is not distributed in a block formation.

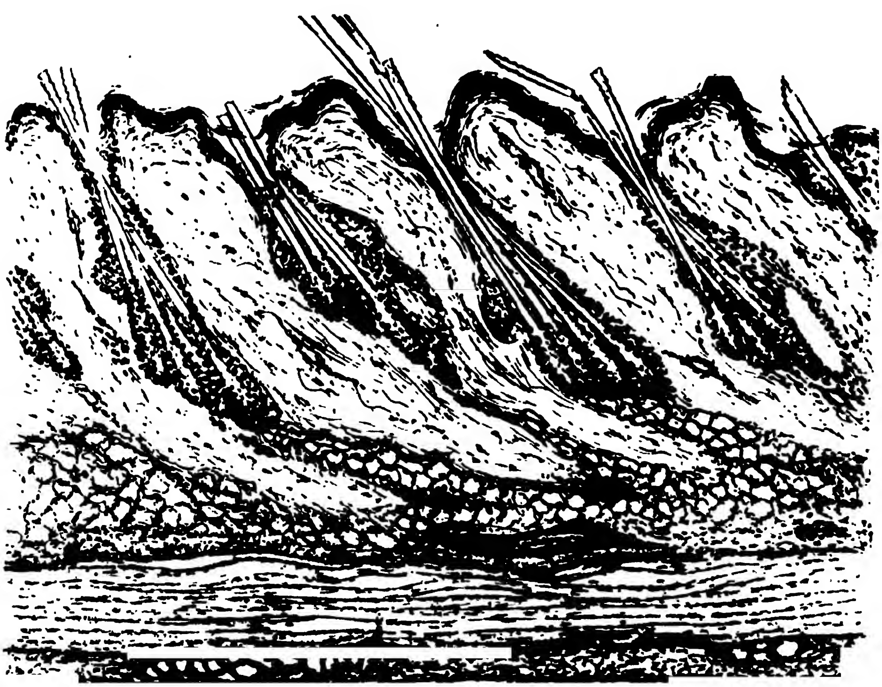
A similar preparation of a prime area shows no hair roots, but only the connective tissue ground substance (Text-fig. 1). The roots are not hidden by an external layer of fibrous tissue, but are invisible because they are completely devoid of melanin.

The blue coloration of the inner layer of the unprime skin which is commonly accepted in the fur trade as indicating unprimeness, is in fact, the massed effect of the pigmented roots. The pink, fleshy color of the prime skin is due to the absence of pigmentation in the hair roots.

It is possible for a muskrat pelt to be as thin as paper, but to be fully prime, and for the skin to be approximately $\frac{1}{8}$ in. in thickness in certain pelts and yet be markedly unprime.

That is to say, primeness or unprimeness is not dependent upon the thickness of the dermis. It is not, as is commonly understood, a pigmented condition of the dermis, but of the pelage, and is due to difference in the distribution of the melanin within the proximal portion of the hairs and their roots.

If vertical sections of a portion of prime skin be examined (Text-fig. 2), the skin is seen to be composed of a relatively thin layer of epidermis, below which lies a thick dermal layer containing the hair roots and follicles with sebaceous glands and arrector pili muscles. Beneath the skin are found the longitudinal muscle fibres of the panniculus carnosus muscle, which is attached by a thin



TEXT-FIG. 2. *Transverse section of prime muskrat skin.* × 55.

layer of fascia to the body musculature. This muscle, although an important constituent of the pelt, is not a "skin muscle" but is a derivative of the pectoral musculature, and is innervated by the nn. thoracales anterior branches of the brachial plexus (7).

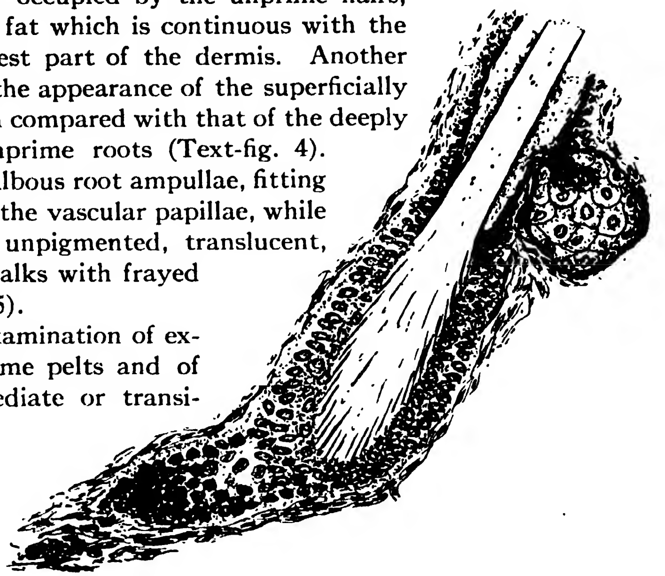
TEXT-FIG. 3. *Transverse section of unprime muskrat skin.* × 55.

Sections of unprime skin show several remarkable differences from the prime condition, particularly with regard to the pigmentation of the hair roots, the relative position, angle and depth of the hairs in the dermis, and the appearance of the root-bulbs in relation to the root papillae.

An important difference between prime and unprime skins concerns the relative depth of the hair roots in the dermis. The unprime or pigmented roots originate in the deepest part of the dermis and are arranged at a very acute angle to the skin surface (Text-fig. 3). In the fully prime pelt the hair roots are situated in the outer half of the dermis and are almost vertical

TEXT-FIG. 4. *Guard-hair root from an unprime area of skin.* × 160.

(Text-fig. 2). Below these groups of prime hairs, trains of follicular cavities are seen curving up from the deeper part of the dermis, from a more horizontal plane than that now assumed by the prime hairs. These cavities, from all evidence, were formerly occupied by the unprime hairs, but are now filled with fat which is continuous with the fatty layer in the deepest part of the dermis. Another noticeable difference is the appearance of the superficially placed prime roots when compared with that of the deeply situated, pigmented unprime roots (Text-fig. 4). The latter have more bulbous root ampullae, fitting like inverted cups over the vascular papillae, while the former appear as unpigmented, translucent, very slightly bulbed stalks with frayed extremities (Text–fig. 5).



TEXT-FIG. 5. *Guard-hair root from a prime area of skin.* × *160.*

From an extensive examination of extreme prime and unprime pelts and of sections of the intermediate or transitional stages of primeness, the conclusion may be drawn that the differences represent merely different phases in the life cycle of the hair. The very young hair has a bulbous root, densely pigmented and situated in the deepest portion of the dermis. The pigment is continued out into the young hair as a continuous core which is often seen to end in a tapering point. Frequently the bulbous part of the root makes a sharp loop or is angled at its junction with the remaining portion of the root, which may be due to the young hair following the old follicular cavity or path of least resistance (Plate II, 1).

As this hair grows older the pigment becomes less dense and assumes a granular appearance, and often shows irregular distribution at the basal extremity. Later the root appears to undergo a process of absorption and atrophy in the region just above the papilla and to assume a depigmented, hyaline appearance throughout, as well as shrinking in diameter. Meanwhile the root is migrating outwards, devoid of an ampulla, and pulled away from the papilla, which remains in the deeper part of the dermis surrounded by a remnant of epithelial cells, from which the next hair generation will probably arise.

This maturation or priming process, although it brings about a separation of the hair root from the papilla, does not sever the hair from all vital connection, however, for when such hairs are clipped from the body of an animal which exhibits seasonal color change, this process is discontinued.

Another structure which may play an important role in bringing about the outward migration and assumption of a more vertical position in the group of

hairs when they become prime, is the arrector pili muscle, which in a few sections is seen attached to the base of the follicle of a group of hairs in its new position. The slightly bulbed prime roots are surrounded by individual epithelial follicles at their deeper extremities, but the outer portions of the roots are more compactly wedged into a common follicle surrounded by a definite connective tissue sheath. Owing to this mechanical arrangement, any outward strain on the hairs of the prime pelt only tends to wedge them more firmly.

The Relation of Unprimeness to Moulting

The pelt of the muskrat consists of two kinds of hairs; a soft thick hair comprising the under-fur, and a longer stouter hair, the guard-hairs, or the protective hair, which overlies the under-fur and, as its name implies, protects and also prevents matting of the under-fur.

An examination of the fleshy side of an *autumn pelt*, taken about October 1, will show the ventral and ventro-lateral regions to be prime, but there may be large blue unprime areas in the region of the neck, around the root of the tail and scattered irregularly along the dorso-lateral areas. On examining the fur, the guard-hairs will be found to be plentiful and the under-fur will be dense in the ventral region of the pelt, but on the dorsal region, the guard-hairs may appear relatively long, owing to the fact that the under-fur here has not attained its full length.

A fully *prime pelt*, taken in the latter part of March, while at the peak of primeness, shows the optimum conditions of sheen, color, texture and density of the fur, and on the fleshy side is devoid of pigmentation. This is the period when the pelt is at the apex of condition and from this time on it usually declines in color, sheen, and so forth.

A *spring pelt* shows the initial stages of moulting. Here the guard-hairs are beginning to shed, especially from the ventral region. The fleshy surface however shows no sign as yet of pigmentation, but does show a marked change in color from a bloody or fleshy color as seen in autumn or winter, to a whitish color. This is due to a decreased vascularity and a deposition of fat on the dermal and fascial sides of the panniculus carnosus muscle.

Pelts taken in the early summer show a slight amount of matting of the under-fur, especially where the old guard-hairs have completely disappeared, and where the young ingrowing guard-hairs are not as yet very long. The under-fur constitutes the main portion of the coat of the animal at the season when the guard-hairs are noticeably absent. But here there is also evidence of early moulting of the under-fur in the ventral regions. The fleshy side of the pelt at this season shows a dense pigmentation owing to the presence of heavy deposits of melanin in the roots of the young ingrowing hairs. The pigmentation at this time is most dense in the ventral region and least dense along the mid-dorsal line, owing to the ingrowth of young under-fur hairs, and of guard-hairs, reaching the maximum concentration first in the ventral portions of the pelt (Plate I, 1).

FIG. 1. *Summer pelt showing mid-dorsal area prime, i.e., converse to winter pelt.*

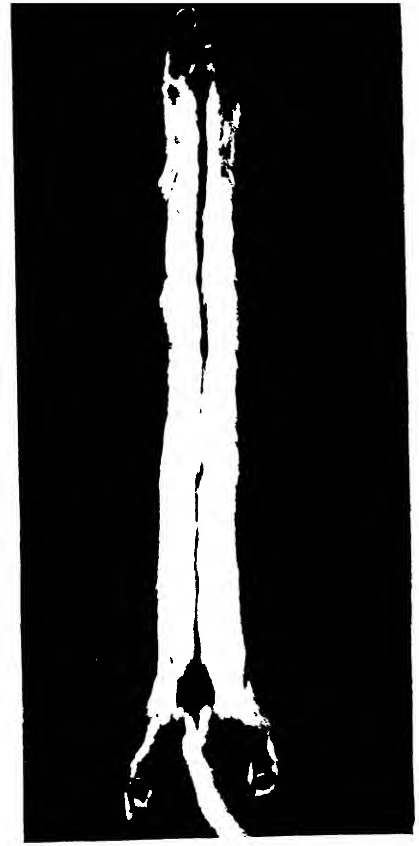


FIG. 2. *Weasel pelt in final stages of blanching process.*

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FIG. 1. *Microphotograph of unprime pelt.* ×78. FIG. 2. *Microphotograph of proximal ends of underfur hairs from an unprime area of pelt.* ×375. FIG. 3. *Microphotograph of proximal ends of underfur hairs from a prime area of pelt.* ×375 FIG. 4. *Microphotograph of transverse section of skunk pelt, without panniculus carnosus muscle.* ×16.

An examination of pelts taken later in the summer shows evidence of moulting as before, but not in the same regions. Now, the shedding process is confined more to the sides and back of the animal and affects the under-fur of these regions. In the ventral regions the young guard-hairs are in evidence and also the short under-fur of the new coat.

In conclusion, to summarize the information available concerning the relation between moulting and unprimeness, and the new growth of fur, it is quite evident that the shedding process does not take place simultaneously in the guard-hairs and under-fur, nor does it occur uniformly throughout the different regions of the surface of the body, but takes place over the various body areas at different intervals of the moulting season and follows a definite sequence, namely:—

(1) First the guard-hairs are shed from the ventral regions of the body surface, and then the under-fur. This process, in this order, then spreads to the lateral and finally into the dorsal surfaces of the trunk of the animal.

(2) First to appear in the new coat are the guard-hairs in the ventral region, then the under-fur. Thus it is evident that the ingrowth of the new coat follows the sequence of the moult.

The last place to become fully furred is the dorsal portion of the body surface and here first the guard-hairs and later the under-fur grow into the new coat. Thus the animal has an adequate coat of fur and at no time is completely devoid of protection, whether it be from the rigors of winter or the heat and actinic rays of summer.

Seasonal Changes in the Appearance of the Leather

The seasonal changes in the character of the fleshy side of a pelt vary according to the time of the year in which the animal is pelted. The fleshy side of an autumn and winter pelt is red in color owing to the presence of a copious blood supply to the panniculus carnosus muscle and underlying connective tissue, but the fleshy appearance gives place to a blue-black pigmentation, limited to several large well-defined unprime areas in the lateral and dorsal regions, especially evident in the skin from the back of the neck along the mid-dorsal line and above the root of the tail. These are the last areas to become prime and may even show evidence of pigmentation in late winter muskrat pelts, but usually they have reached the prime state by the end of March or April.

When animals are trapped in the late spring of the year, the color of the inner surface of the pelt is seen to be changing from red to white, owing to the diminishing blood supply to the pelt, and to the deposition of fat in the skin. Although the guard-hairs at this season pull out more easily, there is as yet no sign of pigmentation in the dermis. Later in the spring the pelt becomes dark red and feels thick, greasy and board-like on the leather side when cased, and the fur is found to be scanty in amount. This kind of a pelt is known in the fur trade as "springy".

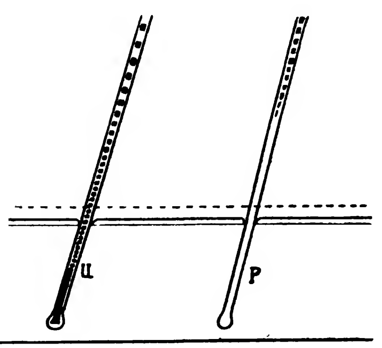
Finally, if pelts taken in mid-summer are examined, they are found to be blue on the ventral surface owing to the very dense pigmentation of the roots in the new ingrowing young hairs. At this season the densest pigmentation is seen in the ventral areas of the pelt. The initial stages of pigmentation are seen in the shoulder areas, thence extending posteriorly along the ventrolateral aspects of the thorax, abdomen and covering the perineum (Plate I, 1). At a slightly later period of the summer this black coloration is visible on the sides and back on the body surface, but unlike the autumn condition, the pigmentation is speckled over this region.

Thus the value of a pelt may be judged by the color of the inner surface, since this is merely an expression of the phenomena taking place in the fur and therefore is indicative of its condition.

The Detection of Primeness or Unprimeness in the Living Animal

The principle of this test is based upon facts obtained by microscopic study of prime and unprime sections. The different areas of the skin surface of an animal, however, become prime at different times of the year and remain at the peak of condition over a short period of the season. The order of priming in a pelt is as follows:— (1) the ventral regions; (2) the lateral regions; (3) the dorsal regions.

The last portions of the dorsal area to become prime are those at the back of the neck and just anterior to the root of the tail. It has been pointed out that the guard-hairs in the new coat appear first and the under-fur comes later, so that the last fur in the muskrat to become prime is the under-fur of these dorsal regions.



TEXT-FIG. 6. *Diagram showing distribution of pigment in (P) prime and (U) unprime hairs.*

A study of vertical sections, and especially of the hair side of the pelt, with a binocular microscope will show that depigmentation of the hair roots does not stop at the level of the epidermis, but is continued out into the hair to a variable extent in different animals (Text-fig. 6). If the hair be examined a short distance out from the epidermis the pigment is seen to return, at first in small irregular groups of melanin granules in the medulla, but which soon assume the characteristically blocked arrangement common to the outer portions of all the under-fur hairs.

The detection of prime from unprime portions of a pelt therefore simply requires the microscopic examination of samples of under-fur hairs cut close to the skin, and an examination particularly of the root ends of these. The hair from the unprime pelt (Plate II, 2) shows pigmentation of a heavy blocked type (discontinuous medulla) extending down to the cut end, while

the hair from a prime pelt shows as a clear hyaline structure, devoid of pigmentation for a variable distance from the cut end (Plate II, 3). The difference is very obvious in hairs derived from prime and unprime areas of a pelt.

The distribution of the pigment thus serves as an exact external indicator of the condition of the skin in estimating the relative primeness of different animals which may show wide variation in time of reaching the peak of this condition, owing to age, sickness, or other natural causes. The test is applicable to a wide range of fur-bearing animals with the exception of albinos, but probably lends itself to more practical application in the fox ranching industry.

Practical Application

The animal is held whilst the fur at the back of the neck is wetted and parted. Then, by means of a straight-edge razor a small lock of under-fur is shaved off close to the skin. The lock is picked up by forceps just behind the former point of attachment to the skin and the outer portion is twisted in order to hold the root-ends together. This facilitates their examination in the dry state under the low power of the microscope. A sample which shows all the root-ends devoid of pigmentation indicates primeness, while the presence of pigmented root-ends even among prime hairs means that the skin from which they originated is not fully prime. This procedure is then repeated with samples taken along the mid-dorsal line and from the region anterior to the root of the tail. Then from the study of the sequence of primeness it is evident that if the samples taken from these areas are prime, the rest of the pelt is already prime.

Discussion

It is evident, from an histological examination of pelts taken from the animal at different seasons of the year, that many features such as unprimeness or pigmentation in the leather, lack of pigmentation of prime pelts, shedding of the fur, etc., are merely the outward expression of changes taking place in the hair and its root during the different phases of the life cycle. Pigmentation is present in the actively growing young hairs which are in direct communication with the vascular papillae, whilst primeness is associated with depigmentation in the root and hair shaft, in the latter to a variable distance in different animals, save in albinos (4). This blanching process is probably seen in its most exaggerated form in such animals as the varying hare, jack rabbit, weasel and arctic white fox, in which it would appear that primeness means, not only depigmentation of the root and proximal part of the hair, but also of the outer hair shaft. This view would account for most of the facts put forward in a recent paper on color change in *Lepus americanus* (5) which asserts that: "Probably the most convincing proof that the change takes place in existing hairs is to be found in the skin itself when the hair roots are examined. The fact that the roots cease to function as the hair turns white, and that it is a progressive change, offers conclusive evidence that the alteration is destructive."

There are objections to this view that seasonal color change in rabbits, etc., is due to a depigmentation process in the roots, since it is evident from the

study of primeness in other colored fur-bearing animals, such as the muskrat, that the roots here also become depigmented when the prime state is reached, without the outer fur blanching. It may be suggested in fact that the seasonal depigmentation, with the consequent color change is merely an exaggerated state of a priming process which occurs annually in all fur-bearing animals, to a variable but lesser extent (excluding albinos). Further evidence supporting this view is found in the fact that the sequence followed in the priming and blanching processes is the same. Thus, not only are the areas at the back of the neck and root of the tail the last to become prime, but also to change color, in variable animals (Plate I, 2).

The impression that the hairs pull out more easily from an unprime pelt than from a prime pelt is a mistaken interpretation of the facts, although it is stated thus by fur-dressers. From the study of the relation of shedding to unprimeness in pelts it is evident that young or pigmented hairs are present where shedding is in progress, and it has been wrongly supposed that these are the hairs which shed more readily. As a matter of fact, as shown by sections of unprime skin, these hairs are more deeply embedded in the dermis and exhibit a definite ampulla firmly attached to the root papilla which anchors them even more firmly in the skin than the prime hairs, which have partly lost this attachment. The hairs which pull out more easily represent a remnant of the former coat which is in the process of shedding in this unprime area.

Another defect which cannot wholly be attributed to unprimeness is that, when unprime pelts have passed through the tanning and dressing processes, the hairs often project on the fleshy side of the leather. This may be the result of improper handling.

In the scraping, the carnosus muscle may be detached from the skin and partly or wholly removed; then, since the hair roots are embedded in the deeper part of the dermis in an unprime pelt, and the tanning process removes the fat in the deepest layer from around the roots, they become exposed and project through the inner surface of the leather (Plate II, 4).

Another cause of this condition is the "fleshing" process. Since the hair roots are so near the inner surface of the leather in the unprime pelt, they may be cut off during this process and owing to the serrate edges of the hair cuticle which project towards the external surface, the hairs tend to work towards the inner side of the leather, when the pelt is subjected to the further manipulation necessary in the dressing process.

In view of the great importance attributed to primeness not only in the grading of furs from the aspect of their consequent money value, beauty and durability, but from the stress laid upon this condition in the legal interpretation of the Game Act, a clear and definite standard of what constitutes primeness and unprimeness is of due importance.

The test for primeness permits the use of the hair shaft as an external indicator of the invisible underlying changes taking place in the skin of the *living* animal, not only as an indicator of the prime condition, but of the influence of varied diets in the production of optimal coloration and their forcing or retarding effect upon the onset of primeness.

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References

1. ALLEN, J. A. On the seasonal change of color in the varying hare (*Lepus americanus* Erxl.) Bull. Am. Mus. Nat. Hist. 6: 107-128. 1894.
2. AUSTIN, W. E. Principles and practice of fur dressing and fur dyeing. Van Nostrand. 1922.
3. DAWSON, H. L. A study of the hair-growth on guinea pig (*Cavia cobaya*). Am. J. Anat. 45: 461-484. 1930.
4. FRASER, D. A. The winter pelage of the adult albino rat. Am. J. Anat. 47. 1931.
5. HADWEN, S. Color changes in *Lepus americanus* and other animals. Can. J. Research, 1: 189-200. 1929.
6. HAUSMAN, L. A. Micrological investigation of the definite hair structure of the Monotremata. Am. J. Anat. 1920.
7. HAUSMAN, L. A. Structural characteristics of the hair of mammals. Am. Naturalist, 54: 496-523. 1920.
8. HAUSMAN, L. A. Further studies of the relationships of the structural characters of mammalian hair. Am. Naturalist, 58: 544-557. 1924.
9. HAUSMAN, L. A. Mammal fur under the microscope. Nat. Hist. 20: 434-444. 1920.
10. HAUSMAN, L. A. The microscopic identification of commercial fur hairs. Sci. Monthly, 10: 70-78. 1920.
11. HAUSMAN, L. A. Hair coloration in animals. Sci. Monthly, 12: 215-222. 1921.
12. LANGWORTHY, O. R. A morphological study of the *Panniculus carnosus* and its genetical relationship to the pectoral musculature in rodents. Am. J. Anat. 35-36. 1925-26.
13. WELCH, F. H. Observations on *Lepus americanus*, especially with reference to the modifications in the fur consequent on the rotations of the seasons, and the change of color on the advent of winter; based on specimens obtained in the province of New Brunswick, North America. Proc. Zool. Soc. Lond. 228-236. 1869.

STUDIES OF POLYMERS AND OF POLYMERIZATION.

VI. THE VULCANIZATION OF METHYL RUBBER[1]

By George Stafford Whitby[2] and Morris Katz[3]

Abstract

Samples of synthetic rubber prepared by the polymerization of dimethylbutadiene at room temperature and at 45° C. respectively were subjected to vulcanization tests in comparison with natural rubber. In an accelerated gum stock containing 3% sulphur the cold polymer gave at best vulcanized products less than one-third as strong and only about one-third as extensible as natural rubber; the heat polymer gave products as extensible but only one-tenth as strong as natural rubber. The incorporation of carbon black greatly increased the strength of the synthetic rubbers, rendering both about half as strong as natural rubber in a similar stock. The vulcanized synthetic rubbers were less "snappy" than natural rubber at room temperature. Increase of temperature improved their speed of retraction, but seriously reduced their breaking strength. Products from the cold polymer showed a greatly increased stiffness and strength at 5° C. as compared with room temperature, and at about 1° C. were non-retractible. In general the synthetic rubbers were much more sensitive than natural rubber to change of temperature. A 50:50 mixture of the heat and cold polymers was also subjected to tests.

The ability of 2, 3-dimethylbutadiene to undergo polymerization to a rubber-like product was first observed by Kondakov (8). When, during the war, the manufacture of synthetic rubber on a large scale was, owing to the exigencies of the time, undertaken in Germany, the polymerizant chosen was 2, 3-dimethylbutadiene, and, since this is a methyl homologue of isoprene, the polymerizant of natural rubber, the rubber produced was designated methyl rubber. About 2350 tons of methyl rubber was manufactured at Leverkusen during the war, and at the close of the war there was in course of erection two additional plants for its manufacture with a joint capacity of 8000 tons a year (4 p. 214, 6). Accounts of the German wartime experience with methyl rubber have been given by Gottlob (5), Duisberg (3), F. Hofmann (6), Burgdorf (1) and Weil (10), but the descriptions of the properties of methyl rubber given by these writers are in general terms only, and, with the exception of Pohle's work on the colloidal behavior of this rubber (9), there is in the literature a lack of any numerical or other exact data concerning the properties of methyl rubber, especially after vulcanization, which would enable them to be compared in quantitative terms with the properties of natural rubber. In view of this lack and of the renewed interest in synthetic rubber which has been excited by the discovery of a synthetic rubber from 2-chlorobutadiene (2) the present work was carried out.

In an earlier paper of this series data have been given on the rate of polymerization of dimethylbutadiene under the influence of heat and on the molecular weight and viscosity of the resulting methyl rubber (12). In the present paper are recorded experiments on the vulcanization of methyl rubber and the properties of the vulcanized products as compared with the properties

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of vulcanized natural rubber. The experiments were confined to soft vulcanized rubber, partly because the statements in the literature relating to the German wartime experience are more definite in regard to the preparation of hard, than in regard to the preparation of soft, rubber from methyl rubber, and partly because the amount of material available was limited. There was used in the experiments methyl rubber of two types, namely (*a*) rubber formed by spontaneous polymerization at room temperature; (*b*) by polymerization at 45° C. These are referred to as the cold polymer and heat polymer respectively and may be compared roughly with the *H* (hart) and *W* (weich) rubbers formerly manufactured in Germany by polymerization at 30° and 70° C. respectively.

In the experiments carried out, the methyl rubbers obtained by cold and by hot polymerization and also a 50:50 mixture of these products were compared with natural rubber in a gum stock and in a carbon black stock. The influence of temperature on the tensile strength, elongation and retractive power of the vulcanizates was also studied.

It was found that in a gum stock the cold polymer gave, at the best cures, vulcanized products less than one-third and the heat polymer not more than one-tenth as strong as were given by natural rubber. The vulcanized cold polymer suffered rupture at a low elongation, the ultimate extension being only about one-third that of natural rubber. The vulcanized heat polymer on the other hand, although, as already stated, far weaker than vulcanized natural rubber, was almost as extensible as the latter (see Table I).

The incorporation of carbon black exercised a much more striking effect in methyl than in natural rubber, particularly in the case of the heat polymer. The addition of 25 parts of this ingredient to the gum stock raised the maximum tensile strength from 28.2 to 166.5 kg. per sq. cm. in the case of the heat polymer; from 86.4 to 161 kg. per sq. cm. in the case of the cold polymer, and only from 293 to 349 kg. per sq. cm. in the case of natural rubber. The profound effect of carbon black on the tensile properties of methyl rubber is shown, not only by the increase in the ultimate tensile strength just noted, but also by the marked stiffening of the stock. The cold polymer, already somewhat stiffer than natural rubber in the gum stock, was, by the addition of carbon black, rendered far stiffer than natural rubber in a similar stock, the load required to extend to twice the original length samples cured to the point of maximum tensile strength being 54.2 and 19.7 kg. per sq. cm. in the two cases respectively (see Table I). Increase in the carbon black from 25 to 50 parts did not, in experiments with the cold polymer, further increase the ultimate tensile strength, although it stiffened the rubber compound, as is indicated in Table I by the figures for T_2 (load producing an extension of twice the initial length). The effect of carbon black in raising the tensile strength of synthetic rubber has lately been mentioned in the patent literature (7).

In view of the relatively small influence of carbon black on natural rubber as compared with its great effect on the soft, heat polymer, it may be surmised that in natural rubber only a very small fraction if any of it is soft material in a relatively low state of polymerization.

As other papers (13, 14) in this series show, high polymeric products are in general markedly heterogeneous and consist of mixtures of polymeric molecules representing a range of molecular weights. Methyl rubber produced by heat polymerization was shown to be thus heterogeneous (12), and it seems fair to assume that the cold polymer too is heterogeneous. Further, it has been shown that, other things being equal, the lower the temperature at which the polymerization of a given polymerizant occurs, the higher is the mean molecular weight of the polymerizate. Hence it is probable that methyl rubber produced by polymerization at room temperature ("the cold polymer") has a higher molecular weight than methyl rubber produced by polymerization at 45° C. ("the heat polymer"). The behavior of the two materials towards solvents and swelling agents, as recorded in the experimental part, is in accord with this view. The heat polymer disperses much more readily than the cold polymer; indeed the latter failed to disperse in all the liquids employed and it was in consequence impossible to determine its molecular weight.

In the case of polystyrene it has been established (15) that the elastic properties of samples prepared in different ways depend on the molecular weight. Material obtained by the spontaneous polymerization of styrene at room temperature has a much higher molecular weight than material obtained by polymerization at *e.g.*, 140° C., and, correspondingly, disperses less readily in solvents and has different elastic properties. The cold-polymerized material has a greater strength and smaller extensibility than the heat-polymerized material. This is analogous to the behavior of the vulcanized products from the samples of methyl rubber obtained in the cold and at an elevated temperature.

It may provisionally be considered that in order to secure optimum elastic properties in an elastic polymer, *i.e.*, high tensile strength together with high extensibility, it is necessary that the polymeric molecules present shall extend over a certain optimum range and shall be present in appropriate proportions. In order to secure stress-strain relations similar to those exhibited by natural rubber, it would seem that, speaking broadly, there should be present a suitable proportion of material in a relatively low state of polymerization in order to render the material readily extensible on the application of small loads; a suitable proportion in an intermediate state of polymerization and responsible largely for the intermediate part of the stress-strain curve, and a suitable proportion in a high state of polymerization which confers tenacity, enables high loads to be sustained with little deformation and is responsible largely for the last part of the stress-strain curve.

In view of considerations such as the foregoing, experiments were carried out on the vulcanization of a mixture of equal parts of the cold and heat polymers. The former polymer, representing material in a higher state of polymerization, yields on vulcanization alone a product of relatively good strength but poor extensibility; the latter, representing material in a lower state of polymerization, a product of poor strength but good extensibility. The product from the 50:50 mixture was found to have almost as good an extensibility as the heat polymer alone and a tensile strength between that of the heat and cold polymer.

The amount of material available did not permit of experiments on mixtures in other proportions, but such further experiments would probably be instructive and are to be desired.

The vulcanized products from methyl rubber not only had a markedly lower tensile strength than those from natural rubber but much poorer "nerve"; that is to say, when allowed to recover under no-load after being extended, they retracted with much less "snap" than natural rubber. This was particularly so with the cold polymer, which retracted very sluggishly and was decidedly loggy. The figures for "set" given in Table I represent the residual extension in per cent half an hour after the specimens had been stretched to the point of rupture. These figures do not well reveal the lack of "snap" in the synthetic rubber. The latter is however well shown by the figures given in Table II for the residual set left at intervals up to four minutes after stretching specimens to 150% and then allowing them to retract under no-load. It was observed that when warmed the specimens of methyl rubber largely lost their sluggishness. This is shown by the data given in Table II on the retraction of the specimens when allowed to retract at 45° C. after being stretched 150% at that temperature. At 70° C. the samples of vulcanized methyl rubber retracted with almost as good a "snap" as natural rubber, but, as data in Table III show, were lacking in strength.

The effect of introducing small proportions of plasticizers into the stiff, carbon black stock from the cold polymer was examined. It was found to increase somewhat the ultimate extension (Table I) and to improve, but not strikingly, the speed of retraction (Table II).

The tensile properties of methyl rubber vulcanizates were determined not only at room temperature, but also at a reduced temperature (5° C.) and at elevated temperatures (37° and 70° C.). (See Table III.) It was found that all the specimens of vulcanized methyl rubber were much more sensitive to temperature changes than was natural rubber. The heat polymer was particularly sensitive to fall of temperature and the cold polymer to rise of temperature. Reduction in the temperature from 20° to 5° C. increased the tensile strength of the gum vulcanizate from the cold polymer from 28.2 to 181 kg. per sq. cm. Increase in the temperature from 20° to 37° C. reduced the tensile strength of the gum vulcanizate from the heat polymer from 68.4 to 10.9 kg. per sq. cm. Natural rubber on the other hand showed little stiffening when the temperature was lowered to 5° C. and relatively little softening when it was raised to 37° C., the figures for tensile strength at 20°, 5° and 37° C. being 285, 272 and 219 kg. per sq. cm. respectively. Even at 70° C. the natural rubber retained a substantial amount of its tensile strength, especially when it was compounded with carbon black, whereas methyl rubber was extremely weak. Thus, for example, the gum polymerizate from the cold polymer, which at 20° C. had a tensile strength of 68.4 had at 70° C. a tensile strength of only 2.4 kg. per sq. cm.

In conclusion it may be remarked that methyl rubber is certainly susceptible to being influenced in its rate of vulcanization by an accelerator such as is effective with natural rubber. Several attempts to vulcanize a simple mixture

of 100 parts methyl rubber (cold polymer), 5 parts sulphur and 5 parts zinc oxide at 133° C. were entirely unsuccessful. The mixture was quite uncured and stuck to the mould after seven hours' heating. On the other hand, as the data in Table I show, a mixture of the rubber containing only three parts of sulphur but in addition one part of an accelerator of the dithiocarbamate class was well vulcanized after 30 min. heating at 110° C. It may be noted further that the methyl rubbers are much less susceptible to overcuring than is natural rubber. Thus, for example, in the gum stock used natural rubber is well cured in 15 min. at 110° C. and overcured in 20 min. or less at 133° C., whereas methyl rubber (cold polymer) gives similar results on the one hand in 30 min. at 110° C. and on the other hand in 80 min. at 133° C.

Gottlob (5) has stated that in the early experiments with methyl rubber it was found that vulcanization in the presence of sulphur and fillers only led to combination of not more than a few tenths per cent of sulphur, but when heated with 10% of sulphur for one hour at 152.4° C. (4 atm. steam pressure), in the presence of piperidine "a few per cent of sulphur were combined". In the present experiments on the vulcanization of methyl rubber in a stock containing a very strong accelerator combination, *viz.*, a dithiocarbamate with zinc oxide, it was found that the rate of combination of sulphur was of the same order of magnitude as, albeit a little slower than, with natural rubber, and that the heat polymer combined with sulphur a little more slowly than the cold polymer. Data are given in Table V.

Vulcanized methyl rubber was found to imbibe swelling agents to a similar extent to natural rubber (Table IV).

Experimental

The methyl rubber used in these experiments was obtained as follows. Dimethylbutadiene was heated in sealed glass tubes at 45° C. for 186 days. This caused the polymerization of a part of the diene and yielded a viscous liquid. The contents of the tubes were then allowed to stand at room temperature for four summer months, and when the tubes were then examined it appeared the whole of the contents had undergone polymerization. Probably the rapidity with which the diene, still unchanged at the end of the heating period, had polymerized at room temperature was due to "seeding" by the polymer already formed. The contents of the tubes consisted of two clearly distinct parts, namely, a clear, elastic lower portion representing the result of heat polymerization and an upper portion which almost filled the rest of the tube and consisted of the typical white "cauliflower" masses characteristic of cold-polymerized dimethylbutadiene. The heat polymer was about 28% of the whole. The tubes were allowed to stand for three and a half years in all; they were then opened, the two portions were separated and were then employed in the following experiments with as little delay as possible, being kept in an atmosphere of carbon dioxide in the meantime.

Since both the "heat" and the "cold" polymers appeared to be susceptible to oxidation (a few days' exposure to the air caused them to become sticky) an

antioxidant was added to all compounded stocks. Anhydro-aldol-α-naphthylamine proved to be effective in this connection. It prevented oxidation of methyl rubber in compounds kept uncured for at least six weeks.

On the mill the behavior of the cold polymer was noticeably different from that of natural rubber. It was at first crumbly and fell through the rolls, but by continued treatment on hot rolls it became more plastic, and eventually

TABLE I

PHYSICAL PROPERTIES OF NATURAL AND METHYL RUBBER AT 20° C. IN GUM AND CARBON BLACK STOCKS

Cure	Gum stock*						Cure	Gum stock+25 pt. carbon black +1 5 pt. stearic acid				
	T_2	T_5	T_6	T_B	E_B	Set		T_2	T_5	T_B	E_B	Set
I. *Smoked sheet*												
5′/110° C.	—	---	—	3	—	---	15′/110° C.	9.2	85.6	155	606	12.3
10	6 2	19.9	33.7	168	875	10 5	20	13.1	133.5	251	636	18.9
15	10.7	41.2	85.3	274	834	16.4	25	17.5	196	273	580	25.8
20	12.2	62 8	134.0	282	754	22.5	30	19.7	194	349	650	30.3
25	15.1	82.8	172	277	709	24.7	30	19.3	193.5	346	648	39.0
30	16.1	96.8	194	293	710	25 4						
20′/133° C.				24	206							
40				16.4	164							
60				15.5	161							
II. *Methyl rubber* (heat polymer)												
20′/110° C.		10.9		16.8	788	2 2	40′/110° C.		93.2	150.7	611	10.0
30		17.1		21.5	610	1.0	60		150.0	166.5	531	12.3
40		26 2		28.2	509	1.0						
20′/133° C.		---		28 2	365	1.1						
III. *Methyl rubber* (cold polymer)												
30′/110° C.	26.6			77.6	293	2.7	20′/133° C.	55.4		155	340	9.2
20′/133° C.	25.0			86.4	243	1.1	20	54.2		161	302	10.0
40	25.4			68.4	228	1.1	40	90.0		153	278	10.5
60	24.6			81.0	241	1.0						
80	22.0			76.1	245	1.2	Gum stock+50 carbon black+1.5 stearic acid					
							20′/133° C.	101		129	258	12.6
							40	133		156	259	22.4
							60	123		130	217	19.1
						(+ 5% plasticizer)	20′	87		153	350	16.9
						(+10% plasticizer)	20′	65		122	355	15.7
IV. *Methyl rubber* (heat and cold polymers, 50:50)												
30′/110° C.		17.5		33.3	610	2.7						
40		31.0		42.0	535	2.7						
20′/133° C.		—		51.0	400	2.2						

**Gum stock:– 100 pt. rubber, 3 pt. sulphur, 5 pt. zinc oxide, 1 pt. anhydro-aldol-α-naphthylamine, 1 pt. piperidinium pentamethylene dithiocarbamate.*

Tensile strength given in kg. per sq. cm.; elongations and set in per cent. T_2, T_5 T_6 = tensile strength at 200, 500 and 600% elongation respectively. T_B = tensile strength at break; E_B = per cent elongation at break.

there was obtained a sheet, which however was leathery and devoid of tack. The cold polymer took about twice as long to mill as natural rubber. The heat polymer broke down on the mill quite readily and in a similar manner to natural rubber, but a sheet of compounded stock from the heat polymer was noticeably less tacky than a corresponding sheet from natural rubber.

Vulcanization was carried out in moulds designed to produce moulded ring test pieces. These moulds, which were designed by D. F. Stedman, and will shortly be described by him in this Journal, do not involve any waste of rubber in producing test pieces and are well adapted for working with small quantities of material. Strips about 14 cm. long and weighing about 1.5 gm. were cut from the milled sheets and placed in the ring moulds. The latter were then placed in a small hydraulic press and a pressure of 1000-4000 lb. per sq. in. applied for 10-15 min. in order to make the strips fit the moulds perfectly. Vulcanization was then brought about by heating the moulds in a constant-temperature bath at either 110° or 133° C. for various periods of time. The moulded test rings thus secured were of standard diameter (44.5 mm.) and thickness (3.15 mm.) and of a height which varied slightly according to the amount of rubber used but was approximately 2.5 mm. The tensile strength and elongation of the test pieces were determined 24 hr. after curing by means of a Schopper machine which gave an autographic load-strain curve. Set was determined by measuring the length of the test pieces 30 min. after rupture. The set as thus determined is substantially all permanent set, as measurements of test pieces several days after rupture did not indicate any further retraction and as heating to 68° C. for five minutes also failed to produce further retraction.

The results are given in Table I, the figures given for the stocks containing natural rubber represent the mean of the results for three test pieces in each case; those for the methyl rubber stocks in most, but, owing to the limited amount of material, not all cases represent the mean of the results for two test pieces.

The plasticizer used in certain stocks as noted in the table was methylcyclohexyl adipate.

Retraction of methyl rubber. The results given in Table II show the rate of retraction at room temperature (20° C.) and at 45° C. when samples 5 cm. long of various vulcanized stocks from methyl rubber are stretched 150% for three minutes and then allowed to retract under no-load. For comparison, a properly cured and an undercured sample (see Table I) of natural rubber are included. It will be observed that the slow rate of retraction at 20° C. of the methyl rubbers in a gum stock is similar to that of the badly undercured sample of natural rubber.

Effect of temperature on the physical properties of methyl rubber. It has already been pointed out that, although vulcanized methyl rubber, especially if prepared from the cold polymer, is sluggish at room temperature, it becomes readily retractible at elevated temperatures, and at 70° C. or more has a "snap" similar to that of vulcanized natural rubber. Furthermore, if vulcanized methyl rubber is stretched at temperatures below its elasticity temperature,

TABLE II

RATE OF RETRACTION OF VULCANIZED NATURAL AND METHYL RUBBER (GUM STOCK AS GIVEN IN TABLE I)

SPECIMENS EXTENDED 150% FOR THREE MINUTES AND ALLOWED TO RETRACT

Sample	Cure	Set after:				
		0.25 min.	0.75 min.	2 min.	4 min.	30 min.
At 20° C.						
Natural rubber	5′/110° C.	8.0	5.6	3.0	2.2	2.0
Natural rubber	20′/110° C.	2.0	1.2	1.0	1.0	1.0
Heat polymer	30′/110° C.	10.0	4.0	1.0	1.2	0.4
Cold polymer	40′/133° C.	9.0	6.0	1.0	1.0	1.0
Cold polymer+25 pt. C. black	40′/133° C.	22.2	13.0	7.0	5.0	2.4
Cold polymer+50 pt. C. black	20′/133° C.	56.0	29.0	15.0	9.9	4.8
Same+10 pt. plasticizer	20′/133° C.	30.0	16.0	9.0	7.2	3.4
Unvulcanized heat polymer		50.0	35.0	24.0	16.0	10.0
At 45° C.						
Heat polymer	30′/110° C.	10.0	2.4	1.6	1.6	1.6
Cold polymer	30′/110° C.	6.0	2.0	1.0	1.0	1.0
Cold polymer+25 pt. C. black	40′/133° C.	3.6	2.2	1.2	0.4	0.4
Cold polymer+50 pt. C. black	20′/133° C.	8.0	5.6	3.6	2.6	2.6
Same+10 pt. plasticizer	20′/133° C.	9.0	4.4	2.8	1.6	1.2

say 1° C., it will remain stretched and will not retract until warmed. Its behavior in this respect is similar to that of raw rubber. In order to study more closely the effect of temperature on methyl rubber, in comparison with its effect on natural rubber, a series of tensile measurements were made at temperatures of 5°, 20°, 37° and 70° C.

For the purpose of these measurements there was made and mounted on a Schopper testing machine a constant-temperature bath which permitted the specimens to be immersed in water at the desired temperature along its entire length up to the point of rupture. The bath consisted of a long rectangular metal tank, 34 by 3.5 by 4 in., with a hole in the bottom to allow the passage of the rod carrying the lower grip. By means of a packing gland of oil and graphite, the hole was made water-tight. Electrically regulated immersion heaters were inserted in the tank, and the water was stirred by means of a stream of air, which proved more satisfactory than a mechanical stirrer. Repeated tests with ordinary rubber showed that the effect of immersion in water during the short period of the test was negligible. The specimens were immersed without tension for a short time in the water before stretching, in order to allow them to come to temperature. Grips for holding the ring shaped test pieces presented a little difficulty at first, because it was found

TABLE III

THE EFFECT OF TEMPERATURE ON THE STRESS-STRAIN PROPERTIES OF NATURAL AND METHYL RUBBER

	Heat polymer	Cold polymer		Heat and cold polymers (50:50)		Heat polymer+25 pt. carbon black		Cold polymer+25 pt. carbon black	Smoked sheet	Smoked sheet+25 pt. carbon black
Temperature, 5° C.										
Cure	40'/110° C.	60'/110° C.			20'/133° C.			40'/133° C.	20'/110° C.	30'/110° C.
T_3	33.1	36.0			86 6				22.3	72.2
T_5									131	264
T_B	181	64			161			138	272	350
E_B	516	393			411			230	622	593
Set	4.5	6.1			4.7				20.2	35.0
Temperature, 20° C.										
Cure	40'/110° C.	30'/110° C.	40'/133° C.	40'/110° C.	20'/133° C.	40'/110° C.	60'/110° C.	40'/133° C.	20'/110° C.	30 /110° C.
T_3	19.2		25.4	7.8	18.5	32.0	49.0		18.6	48.0
T_5	26.2			31.0					80.8	194
T_B	28.2	77.6	68.4	42.0	51.0	150.7	166.5	153	285	347
E_B	509	293	228	535	400	611	531	278	694	649
Set	1.0	2.7	1 1	2.2	2.2	10.0	12.3	10.5	18 6	34.4
Temperature, 37° C.										
Cure	40'/110° C.	60'/110° C.	40'/133° C.	40'/110° C.				40'/110° C.	20'/110° C.	30'/110° C.
T_3	8.1	6.8	5 0	6.1					17.1	38.7
T_5									48.5	140
T_B	14.8	13.2	10.9	22.3				55.0	219	285
E_B	360	317	280	475				230	712	668
Set	0.9			3.4				5 6	19.5	43.5
Temperature, 70° C.										
Cure	40'/110° C.		40'/133° C.		20'/133° C.	40'/110° C.	60'/110° C.	40'/110° C.	20'/110° C.	30'/110° C.
T_3						15 3	25.4	25.9	15.1	28.8
T_5									36.1	84.9
T_B	6.2		2.4		5.4	23.1	26.3	26.3	106.5	185.5
E_B	230		180		191	380	306	306	680	685
Set	1.1		1 0		1.1			4.5	21.4	

NOTE:— *Stock: 100 pt. rubber or methyl rubber, 3 pt. sulphur, 5 pt. zinc oxide, 1 pt. anhydro-aldol-α-naphthylamine, 1 pt. piperidinium pentamethylene dithiocarbamate.*

that rotation of the test piece was essential for proper results. The required rotation was secured by using at each end of the testing machine gap two ball-bearing pulleys spaced horizontally. The pulleys, which were grooved, were about 1.4 cm. in diameter and about 3 cm. between centres. Using this arrangement, it was found that the test piece rotated as tension was applied, and there was no indication of the rubber being cut at the point of contact. Substantially similar results were obtained in comparative tests using these pulleys in the bath, and then substituting the standard mechanically rotated pulleys of the Schopper machine and carrying out tests at the same temperature in the air.

Selected cures of the same series of stocks (a gum stock and a carbon black stock) as had already been tested at room temperature (Table I) were put through tests at the other temperatures mentioned. The results are given in Table III.

Behavior towards swelling agents. In benzene and in chloroform the cold polymer only partly dissolved after seven days and left a large swollen residue; the heat polymer was nearly all dissolved after two days, although even after seven days there still remained a small undispersed residue. After seven days in ether, the cold polymer was slightly swollen but undissolved; the heat polymer had dissolved to the extent of nearly one-half. The cold polymer swelled in ethyl benzoate and in piperidine, but only a small portion had dissolved after seven days. Nearly one-half of the heat polymer dissolved in these liquids in two days, but it had not all dispersed after seven days. The above observations indicate the mean molecular weight of the cold polymer is markedly higher than that of the heat polymer, and that both materials are composed of a mixture of molecules in different states of polymerization (cf. 11).

The swelling of samples of the methyl rubber and of natural rubber cured under similar conditions in the gum stock was measured with the results given in the following table.

TABLE IV

SWELLING OF VULCANIZED METHYL RUBBER, SYNTHETIC ISOPRENE RUBBER AND NATURAL RUBBER

SAMPLES CURED FOR 30 MIN. AT 110° C. IN THE GUM STOCK QUOTED IN TABLE I

	Gm. liquid imbibed by 0.1 gm.							
	Cold polymer		Heat polymer		Natural rubber		Synthetic isoprene rubber*	
	1 day	2 days	1 day	2 days	1 day	2 days	1 day	2 days
Benzene	0.330	0.3305	0.370	0.371	0.345	0.345	0.330	0.330
Petrolic ether	0.190	0.190	0.210	0.211	0.180	0.180	0.1735	0.175

**In this case isoprene polymer obtained by polymerization in emulsion was vulcanized for 40 min. at 110° C.*

Vulcanization coefficient. Determinations of combined sulphur in comparable cured stocks gave the following results.

TABLE V

COMBINED SULPHUR IN VULCANIZED METHYL AND NATURAL RUBBER

	Combined sulphur as percentage of rubber	
	Cure, 30 min./110° C.	Cure, 20 min./133° C.
Natural rubber	3.09	3.11
Heat polymer	2.42	3.01
Cold polymer	1.89	3.05

Stock: Rubber, 100; S, 3; ZnO, 3; anhydro-aldol-α-naphthylamine, 1; piperidinium pentamethylene-dithiocarbamate, 1.

The total sulphur in the stock was 3.27% (expressed on the rubber). Of this the accelerator contributes 0.27%. Judging from the results for the natural rubber samples it would seem that about 0.17% of sulphur from the accelerators remains extractable.

Acknowledgment

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References

1. BURGDORF, C. C. Ind. Eng. Chem. 18: 1172-1173. 1926.
2. CAROTHERS, W. H., WILLIAMS, I., COLLINS, A. M. and KIRBY, J. E. J. Am. Chem. Soc. 53: 4203-4225. 1931.
3. DUISBERG, C. Z. Electrochem. 24: 369-372. 1918
4. GOTTLOB, K. Technologie der Kautschukwaren, 2nd. ed., Braunschweig, 1925.
5. GOTTLOB, K. India Rubber J. 58: 305-8, 348-50, 391-5, 433-6. 1919.
6. HOFMANN, F. Mitteilungen aus dem schlesischen Kohlenforschungsinstitut des Kaiser-Wilhelm-Gesellschaft in Breslau, vol. II, 235-248. 1925.
7. I. G. Farbenindustrie, Fr. Pats. 655217, 701102. 1928, 1930.
8. KONDAKOV, I. J. prakt. Chem. 64: 109-111. 1901.
9. POHLE, H. Kolloidchem. Beihefte, 13: 1-60. 1921.
10. WEIL, R. Ind. Eng. Chem. 18: 1174-1177. 1926.
11. WHITBY, G. S. J. Phys. Chem. 36: 198-214. 1932.
12. WHITBY, G. S. and CROZIER, R. N. Can. J. Research, 6: 203-225. 1932.
13. WHITBY, G. S. and KATZ, M. J. Am. Chem. Soc. 50: 1160-1171. 1928.
14. WHITBY, G. S. and KATZ, M. Can. J. Research, forthcoming (on polystyrene).
15. WHITBY, G. S., MCNALLY, J. G. and GALLAY, W. Colloid symposium monograph, 6: 225-236. 1928.

THE PREPARATION OF β-ARYLETHYLAMINES FROM α-CYANO-β-ARYLACRYLIC ACIDS[1]

By John Alexander McRae[2] and William Henry Vining[3]

Abstract

Details are given of a method by which β-arylethylamines can be prepared from α-cyano-β-arylacrylic acids. Conditions for the elimination of carbon dioxide from several α-cyano-β-phenylacrylic acids have been studied and, in agreement with the results of others, copper powder proved to be the most useful catalyst among the substances tried. The action of alkaline hypobromite solutions on phenyl- and piperonyl-succinimides resulted only in hydrolysis to the respective succinic acids.

It was shown several years ago by Baker and Lapworth (1) that α-cyano-β-arylacrylic acids can be reduced readily to α-cyano-β-arylpropionic acids and it has been shown further by Baker and Robinson (2) that α-cyano-β-piperonyl-propionic acid thus prepared can be converted smoothly into β-piperonyl-propionic nitrile. They state further that this nitrile is changed easily into the corresponding amide by Radziszewski's method. Prior to the publication of these papers we had carried out somewhat similar experiments, of which an account is now given, starting with several α-cyano-β-arylacrylic acids with the object of testing the feasibility of using the following scheme for the preparation of β-arylethylamines;

$$R.CHO \longrightarrow RCH{:}C(CN)CO_2H \longrightarrow RCH_2.CH(CN)CO_2H \longrightarrow RCH_2.CH_2CN \longrightarrow R.CH_2.CH_2.CONH_2 \longrightarrow RCH_2.CH_2NH_2.$$

To the authors this seems to have certain advantages over the following scheme, which is usually used (Slotta and Heller (9) have summarized recently the applications of this and other methods for preparing β-arylethylamines),

$$RCHO \longrightarrow RCH{:}CHCO_2H \text{ or } RCH{:}CH(CO_2Et) \longrightarrow RCH_2.CH_2CO_2H \longrightarrow RCH_2.CH_2.COCl \longrightarrow RCH_2CH_2CONH_2 \longrightarrow RCH_2CH_2NH_2,$$

in that the amides required for the last step are produced directly from α-cyano-β-arylacrylic acids, and these acids Lapworth and McRae (6), and Baker and Lapworth (1) have shown, are readily accessible substances.

The α-cyano-β-arylacrylic acids employed were those derived from benzaldehyde, anisaldehyde, piperonal, vanillin and veratric aldehyde. In each case excellent yields of the reduced acids were obtained by using as the reducing agent sodium amalgam prepared according to the directions of Raiford and Clark (8). The elimination of carbon dioxide from the α-cyano-β-arylpropionic acids, $RCH_2.CH(CN)CO_2H$, takes place easily by heating the acids with copper powder or preferably quinoline. Hydrolysis of the resulting nitriles,

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[3] *Holder, at the time, of a bursary under the National Research Council of Canada.*

$RCH_2.CH_2CN$, was effected by means of cold, fuming hydrochloric acid, which we prefer to the Radziszewski method. As the result of numerous experiments the writers have been able to obtain uniformly high yields of β-arylethylamines from β-arylpropionamides through the action of alkaline sodium hypobromite which, contrary to the experiences of others, gave in this work better yields than alkaline sodium hypochlorite.

The yields in the various stages based on many experiments are as follows:

TABLE I

AVERAGE YIELDS OBTAINED IN CONVERSION OF α-CYANO-β-ARYLACRYLIC ACIDS INTO β-PHENYLETHYLAMINES

α-Cyano-β-aryl-acrylic acid from	I. Reduction (sodium amalgam), %	II. Elimination of CO_2 (quinoline as catalyst), %	III. Hydrolysis (fuming HCl)	IV. Conversion to amine, (NaBrO+ NaOH), %
1. Benzaldehyde	95	80–83	Almost quant.	66
2. Anisaldehyde	90–95	80–82	Almost quant.	80
3. Piperonal	95	80	Almost quant.	70
4. Vanillin	95	69	——	——
5. Veratric aldehyde	90	75	Almost quant.	70

The proposed method, therefore, gives good yields at all stages. An attempt to prepare histamine by applying this scheme to glyoxaline-(4 or 5)-formaldehyde failed. The authors were unable to bring about the initial condensation of this aldehyde with cyanoacetic acid.

Concurrently, a series of experiments on the elimination of carbon dioxide from α-cyano-β-arylacrylic acids using the acids from benzaldehyde and piperonal showed that of the various catalysts employed, copper powder was the most effective; quinoline with acids of this type promoted resinification to such a marked degree that it was useless for the purpose in mind.

A number of attempts were made to prepare β-aryl-β-carboxylic-ethylamines or, alternatively, β-aryl-β-aminopropionic acids by the action of alkaline sodium hypobromite on aryl-succinimides, using phenyl- and *piperonyl-* succinimides as examples, but hydrolysis to the corresponding acid was the only change observed.

Experimental

A. Reduction of α-Cyano-β-arylacrylic Acids

The α-cyano-β-arylacrylic acids used were prepared by the general method of Lapworth and McRae (6). The crude acids so obtained from the condensation of the aromatic aldehydes with sodium cyanoacetate were washed with benzene and recrystallized from dilute ethyl alcohol.

α-Cyano-β-veratrylacrylic acid was made also by the methylation of α-cyano-β-(3-methoxy-4-hydroxyphenyl) acrylic acid in alkaline solution by means of dimethyl sulphate but the method is less advantageous than that of first methylating vanillin to veratric aldehyde and condensing the latter with sodium cyanoacetate.

As reducing agents (a) sodium amalgam, (b) electrolytic reduction with a mercury cathode, (c) hydrogen in presence of colloidal palladium, and (d) aluminium-mercury couple on the ammonium salt of the acid, were tried. By far the most satisfactory and convenient reducing agent is sodium amalgam, made according to the directions of Raiford and Clark (8). Using amalgam prepared thus, the reduced acids obtained on acidifying the reaction mixture solidified rapidly, whereas with sodium amalgam prepared in the ordinary way crystallization was frequently delayed greatly. In most of these experiments the α-cyano-β-arylacrylic acids were dissolved in N/1NaOH, an equivalent of sodium bicarbonate was added and the reduction carried out at 35° C., using a 25-50% excess of sodium amalgam. This procedure presents no advantage over that used by Baker and Lapworth.

Although from time to time comparatively large amounts of cyanophenylacrylic acid have been reduced in this laboratory, at no time have the writers observed the interesting by-product isolated by Baker and Lapworth in one of their experiments. The α-cyano-β-arylpropionic acids were purified and agreed with the descriptions given by previous authors. The following acid, however, had not been prepared hitherto.

α-Cyano-β-(3-methoxy-4-hydroxyphenyl)-propionic Acid (α-Cyano-β-vanillylpropionic acid)

$$\begin{matrix} (3)\ CH_3O \searrow \\ \quad\quad\quad\quad C_6H_3.CH_2.CH(CN)CO_2H. \\ (4)\ HO \nearrow \end{matrix}$$

α-Cyano-β-vanillylacrylic acid (43 gm.) was suspended in 200 cc. of water. Five hundred grams of $2\frac{1}{2}$% sodium amalgam was added in five portions. Reduction takes place very readily and is marked by the disappearance of the yellow color of the sodium salt of the unreduced acid. On acidification only a small amount of oil separated. The solution was extracted thoroughly with ether and after removal of the ether the residual oil crystallized after standing 24 hr. The yield was 41 gm. After several recrystallizations from toluene, in which it is difficultly soluble, the acid had m.p. 80° C. The acid may be recrystallized conveniently on a large scale from hot water, although with this solvent it tends to form supersaturated solutions. The acid is very soluble in alcohol and ether but dissolves with difficulty in benzene and is almost insoluble in ligroin. Analysis: Calcd. for $C_{11}H_{11}O_4N$: C, 59.73; H, 4.98; N, 6.33%. Found: C, 59.51; H, 4.90; N, 6.55%.

B. Conversion of α-Cyano-β-arylpropionic Acids into β-Arylpropionic Nitriles

The five α-cyano-β-arylpropionic acids investigated readily lose carbon dioxide when heated above their melting points but the loss of carbon dioxide is accompanied at times by the formation of considerable tarry material. A number of comparative experiments showed that while copper powder greatly facilitates the removal of carbon dioxide, producing good yields of the

desired nitriles, yet quinoline brings about the same decomposition still more readily and better yields of the nitriles were obtained. The quinoline used was recovered easily.

1. *Hydrocinnamic nitrile*

This nitrile was obtained by heating 43.2 gm. of α-cyano-β-phenylpropionic acid with 14 cc. of quinoline at 145-150° C. until the evolution of carbon dioxide ceased. After shaking the crude nitrile with dilute hydrochloric acid to remove the quinoline, it was extracted with ether and dried over calcium chloride. After removal of the ether 27.2 gm. of hydrocinnamic nitrile boiling at 138-140° C./15 mm. was obtained.

2. *β-Piperonylpropionic nitrile*

This was obtained by heating α-cyano-β-piperonylpropionic acid (73 gm.) with 16 cc. of quinoline at 170° C. until the evolution of carbon dioxide ceased. The crude nitrile was treated as described above and the pure nitrile obtained as a colorless oil distilling at 174-176° C./15 mm. Baker and Robinson (2) give the boiling point as 186-187° C./20 mm. The yield obtained was 81% of the calculated.

3. *β-Anisylpropionic nitrile*

α-Cyano-β-anisylpropionic acid (47 gm.) was heated with 10 cc. of quinoline in an oil bath at 160° C. until the evolution of carbon dioxide ceased. The nitrile was extracted as described above and after removal of the ether 32 gm. or 83% of the calculated quantity of the pure nitrile boiling at 172-173° C./23 mm. was obtained. Goldschmiedt and Fraenkel (4) obtained this nitrile by heating *p*-methoxyphenyl propionic acid with lead thiocyanate and gave the boiling point as 167° C./15 mm.

4. *β-Vanillylpropionic nitrile, β-(3-Methoxy-4-hydroxy) phenylpropionic nitrile*

$$\left.\begin{array}{l}(3)\ CH_3O \\ (4)\ HO\end{array}\right\rangle C_6H_3.CH_2.CH_2CN.$$

Sixty grams of α-cyano-β-vanillylpropionic acid was heated with 17 cc. of quinoline at 160-170° C. in an oil bath until evolution of carbon dioxide ceased. After treatment as described above the nitrile was obtained as a colorless viscous oil distilling at 189-192° C./11 mm. The yield obtained was 36 gm. or 69% of the calculated. On standing the oil solidified and was recrystallized being obtained in prismatic needles from benzene and also from ether by precipitation with petroleum ether. M.p., 58° C. Analysis: Calcd. for $C_{10}H_{11}O_2N$: C, 67.8; H, 6.21; N, 7.91%. Found: C, 67.72; H, 6.25; N, 8.13%.

The substance produces a transitory sharp, stinging sensation when placed on the tongue. With ferric chloride a blue color is produced which fades rapidly to a dirty brown turbidity.

5. *β-Veratrylpropionic nitrile*

α-Cyano-β-veratrylpropionic acid (70.5 gm.) heated with 15 cc. of quinoline gave off carbon dioxide freely at 130° C. After evolution of the gas slackened the temperature was raised to 170° C. and maintained at that point until the decomposition was complete. The pure nitrile was obtained as before by fractionating under reduced pressure and agreed with the description given by Baker and Robinson. The average yield in five experiments was 75%.

C. Conversion of β-Arylpropionic Nitriles to β-Arylpropionic Amides

The method of Radziszewski (7) for the hydrolysis of nitriles was used successfully by Baker and Robinson (2) in the case of β-piperonylpropionic nitrile and this we have subsequently confirmed, but previously the authors' experience with alkaline hydrogen peroxide applied to phenylpropionic nitrile resulted in no hydrolysis, small yields of amide or complete conversion to the acid. The writers, therefore, discarded the use of that reagent in favor of fuming hydrochloric acid saturated at 0° C. and applied it to the foregoing nitriles. The procedure adopted was to treat the nitrile in a stoppered flask with 1½-2 times its weight of the fuming acid and allow the mixture to stand overnight in the ice chest. The amide which had separated was filtered without diluting on a sintered glass filter, the hydrochloric acid thoroughly removed and the amide recrystallized, if necessary, from the appropriate solvent. The phenyl-, piperonyl-, anisyl- and veratryl-propionamides so obtained had in each case the properties previously described. In general the crude amides were pure enough for immediate use in the Hofmann reaction. The average yields obtained in a series of each experiment with each nitrile were those stated above.

All attempts to prepare *β-vanillylpropionamide* from the corresponding nitrile either by the use of fuming, concentrated or dilute hydrochloric acid or caustic soda, gave either unchanged material or hydroferulic acid.

D. Preparation of β-Arylethylamines

Although alkaline sodium hypochlorite has been preferred by several authors to alkaline sodium hypobromite, our observations indicated that with the former reagent ammonia is more frequently evolved on heating the amide with the reagent than with the latter reagent and the yields of amine lower accordingly. The procedure finally adopted was uniform for each amide used and is illustrated sufficiently by the following preparation.

Preparation of β-Piperonylethylamine (Homopiperonylamine)

Sodium hydroxide (20 gm.) was dissolved in 150 cc. of water and the solution cooled to −10° C. Bromine (19.5 gm.) was dropped in slowly while the solution was stirred vigorously by mechanical means. Piperonyl propionamide (19.3 gm. = 1/10 mole) stirred into a paste with 24 cc. of water was added, using an additional 20 cc. of water for washing. After all the amide had dissolved 40 gm. of powdered sodium hydroxide was added and the solution

heated on the water bath at 85° C. for about 20 min. The amine which separated was extracted with ether, dried and distilled under reduced pressure. The homopiperonylamine had b.p. 145° C./11 mm. and was obtained in 70% yield.

Similarly, β-phenylethylamine, β-anisylethylamine and β-veratrylethylamine were obtained consistently in numerous experiments in 66, 80 and 70% yields respectively. Each of the amines was identified fully.

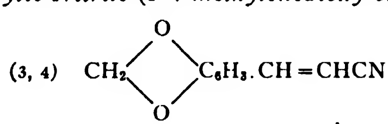
E. Elimination of Carbon Dioxide from α-Cyano-β-phenylacrylic Acids

Fiquet (3) and others have pointed out that arylidene-cyanoacetic acids lose carbon dioxide when heated above their melting points. Preliminary observations on the acid obtained by condensing benzaldehyde with sodium cyanoacetate and purified to a certain extent (a) by washing with benzene or (b) recrystallizing from alcohol, showed that the process of elimination of carbon dioxide was accompanied by considerable resinification. Accordingly the effect of the addition of various substances on this reaction has been studied. Quinoline and diethylaniline promoted resin formation to such a marked degree that any catalytic action on the elimination of carbon dioxide was completely masked. Aniline hydrochloride also promoted resinification. Heating the acid in presence of phosphoric acid and glycerol failed to effect any improvement in yields over those obtained by heating the acid alone. Copper powder on the other hand facilitated greatly the elimination of carbon dioxide and it was applied further for the preparation of β-piperonylacrylic- and β-anisylacrylic nitriles.

(1) *Cinnamic nitrile*

This was obtained by heating recrystallized α-cyano-β-phenylacrylic acid (17.3 gm.) with 34 gm. of copper powder in an oil bath at 180° C. for 30 min. The mixture was distilled in steam, the distillate extracted with ether and after removing the ether the residual oil was distilled under reduced pressure, 7.5 gm. being collected at 115-125° C./15 mm. Yield, 60%.

(2) *β-Piperonylacrylic Nitrile (3-4-methylenedioxy-cinnamic nitrile)*



α-Cyano-β-piperonylacrylic acid (21 gm.) was mixed with twice its weight of copper powder and heated at 185° C. for 30 min. The residue was extracted with ether and the crude nitrile, obtained by removal of the ether, was purified by either recrystallization from alcohol or distillation under reduced pressure, followed by recrystallization from hot alcohol from which it was deposited as a mass of minute colorless needles. M.p., 92° C., b.p., 163-170° C./14 mm. Analysis: Calcd. for $C_{10}H_7O_2N$: N, 8.1%. Found: N, 8.3%.

(3) *Vanillylacrylic Nitrile. 3-Methoxy-4-hydroxy-cinnamic Nitrile*

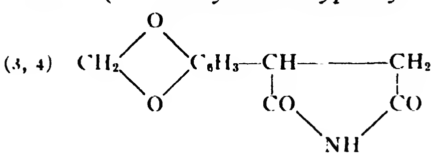
$$\begin{matrix} (3)\ CH_3O \diagdown & \\ & C_6H_3CH{:}CHCN \\ (4)\ HO \diagup & \end{matrix}$$

It was found that copper powder was of relatively small value in eliminating carbon dioxide from α-cyano-β-vanillylacrylic acid. Accordingly the cyano acid was heated rapidly in a Claisen flask under reduced pressure when a colorless liquid, distilling chiefly at 200-225° C./15 mm., passed over and solidified immediately. The crude substance was obtained in 60% yield and was recrystallized from dilute alcohol from which it was deposited in short truncated rectangular prisms. M.p. 112° C. Analysis: Calcd. for $C_{10}H_9O_2N$: N, 8.00%. Found: N, 8.39%. Ferric chloride added to an alcoholic solution of the substance produces an emerald green coloration, which changes on standing to a yellowish brown. A drop of an alcoholic solution of the substance placed on the tongue produces a sharp stinging sensation lasting but a few seconds. It dissolves in sodium hydroxide, forming a yellow solution, and in concentrated sulphuric acid, giving a deep red solution.

F. Action of Alkaline Sodium Hypobromite on Phenylsuccinimide and Piperonylsuccinimide

Although Hoogewerff and Van Dorp (5) obtained β-alanine in yields of 60% by the action of alkaline sodium hypobromite on succinimide, we failed to obtain even traces of any of the possible amino acids which might result from the application of this reagent to either phenylsuccinimide or piperonylsuccinimide. The exact procedure of these authors was used in several experiments. The imides dissolved readily in the alkaline hypobromite solution but, on heating, ammonia was evolved freely and only phenylsuccinic and piperonylsuccinic acids were isolated in good yield, although careful search was made for the expected amino acids. Less strongly alkaline solutions gave the same results. Prolonging the period in which the imide was left in the cold hypobromite solution before heating likewise effected no change. Further experiments on these reactions are in progress.

Piperonylsuccinimide (3-4-methylenedioxyphenylsuccinimide)



Piperonylsuccinic acid (6) was converted into its ammonium salt. The dry ammonium piperonylsuccinate was heated at 150-160° C. until evolution of ammonia ceased. The residue was dissolved in hot aqueous alcohol, which deposited crystals of piperonylsuccininimide on cooling. After several recrystallizations from alcohol the colorless crystals obtained had m.p. 169° C. Analysis: Calcd. for $C_{11}H_9O_4N$: C, 62.1; H, 4.11; N, 6.39%. Found: C, 61.7; H, 4.55; N, 6.57%.

References

1. Baker, W. and Lapworth, A. J. Chem. Soc. 125: 2333-2338. 1924.
2. Baker, W. and Robinson, R. J. Chem. Soc. 127: 1424-1433. 1925.
3. Fiquet, E. Ann. chim. phys. (6) 29: 433-504. 1893.
4. Goldschmiedt, G. and Fraenkel, O. von. Monatsh. 35: 383-390. 1914.
5. Hoogewerff, S. and Van Dorp, W. A. Rec. trav. chim. 10: 4-12. 1891
6. Lapworth, A. and McRae, J. A. J. Chem. Soc. 121: 1699-1712. 1922.
7. Radziszewski, B. Ber. 18: 355-356. 1885.
8. Raiford, L. C. and Clark, E. P. J. Am. Chem. Soc. 45: 1738-1743. 1923.
9. Slotta, K. H. and Heller, H. Ber. 63: 3029-3044. 1930.

STUDIES ON HOMOGENEOUS FIRST ORDER GAS REACTIONS

II. THE DECOMPOSITION OF THE ISOMERIC ESTERS BUTYLIDENE DIACETATE AND ETHYLIDENE DIPROPIONATE[1]

By C. C. Coffin[2]

Abstract

The gaseous decompositions of the esters butylidene diacetate and ethylidene dipropionate have been studied from points of view previously outlined in papers on the decomposition of ethylidene diacetate (2, 3). The decomposition velocities have been measured at initial pressures of from 5 to 56 cm. of mercury and at temperatures between 211 and 265° C. The reactions are homogeneous and of the first order. They agree with the Arrhenius equation and give 100% yields (within experimental error) of an aldehyde and an anhydride. The preparation of the compounds and improvements in the technique of the velocity measurements are described.

While the specific velocities of the three reactions at any temperature are somewhat different, their activation energies are the same. It is suggested that in the case of such simple reactions, which are strictly localized within the molecular structure, the activation energy can be identified as the maximum energy that the reactive bonds may possess and still exist; *i.e.*, it may be taken as a measure of the stability of the bonds which are broken in the reaction. The suggestion is also made that for a series of reactions which have the same activation energy, the specific velocities can be taken as a relative measure of the number of internal degrees of freedom that contribute to the energy of activation. On the basis of these assumptions it becomes possible to use reaction-velocity measurements for the investigation of intramolecular energy exchange. The theoretical significance of the data is further discussed and the scope of future work in this connection is indicated.

The monomolecular velocity constants (sec^{-1}) of the decomposition of ethylidene diacetate, ethylidene dipropionate and butylidene diacetate are given respectively by the equations $\ln k = 23.74 - \frac{32,900}{RT}$, $\ln k = 23.96 - \frac{32,900}{RT}$, and $\ln k = 24.20 - \frac{32,900}{RT}$.

Introduction

During the last few years the kinetics of homogeneous first order gas reactions in general have been so satisfactorily elucidated that measurements of the velocity of simple monomolecular changes have acquired a distinct theoretical significance. The reaction must of course be "simple", *i.e.*, strictly localized within the molecule, before the activation energy can be identified with any particular mechanism or taken as a measure of the stability of the bond or bonds involved. The fact that the majority of the monomolecular reactions so far investigated are too complicated to furnish any detailed information regarding such questions does not detract from their value in clarifying the general mechanism of activation by collision, and in estimating the number of internal degrees of freedom that may contribute to the activation energy. A study of simple first order gas reactions is being carried out in this laboratory in an attempt to establish a connection between bond stability and activation energy, as well as to determine the relations between molecular structure and the number of internal degrees of freedom available for activation.

[1] *Manuscript received February 13, 1932.*
Contribution from the Department of Chemistry, Dalhousie University, Halifax, Canada.

[2] *Associate Professor of Chemistry, Dalhousie University.*

The mechanism by which esters of the general formula $R'CH\langle\begin{smallmatrix}OOCR''\\OOCR'''\end{smallmatrix}$ decompose on heating has been indicated and the break up of ethylidene diacetate in particular has been discussed in previous communications (2, 3). The present paper deals with the decomposition of two isomeric homologues of ethylidene diacetate, *viz.*, ethylidene dipropionate and butylidene diacetate which, like ethylidene diacetate, break up homogeneously by the monomolecular mechanism to give quantitative yields of an aldehyde and an anhydride. This reaction, the mechanism of which appears to remain the same throughout the series of compounds, may thus be expected to lead to interesting data regarding the effect of molecular structure on the rate of chemical change. The simplicity of the reaction, which is essentially the change of an ether oxygen to a carbonyl oxygen, is an important factor in connection with the significance of the velocity measurements.

It has been found that while the specific velocities of these three ester decompositions are slightly different at any temperature, their energies of activation are the same within experimental error. Whether or not this energy (32,900 cal. per mole) is characteristic of the conversion of an ethereal to a carbonyl oxygen or of the breaking of two $-\overset{|}{\underset{|}{C}}-O-$ bonds is being investigated by studying other reactions where the same apparent structural shift occurs. Thus the low temperature gaseous decompositions of the paraldehydes and the acetals have been found to be homogeneous first order reactions, and are being studied with the view of determining the effect of intramolecular environment on the energy necessary to rupture the bonds in question.

With regard to the velocities of the ester decompositions, the working hypothesis is advanced that, since the activation energy is the same in each case, the reaction rate is directly proportional to the number of internal degrees of freedom involved in the activation. Whether or not it will be possible to determine in any instance the actual number of contributory degrees of freedom is not yet clear, as no falling-off of the velocity constant has been observed at pressures as low as 5 cm. of mercury. An attempt is being made to do this in the case of the simplest member of the series, *viz.*, formylidene diformate.

Experimental

Apparatus and Technique

The first 17 runs with butylidene diacetate and thefirst 3 runs with ethylidene dipropionate were carried out in the apparatus already described (3). All the remaining runs were made in a new and improved apparatus of the same general design. The volume of the new reaction tube was made much larger than the first (262.5 cc. as against 120 cc.) in order to obtain increased accuracy as well as sufficient amounts of reaction products for analysis. To avoid possible cooling of the reaction chamber by the reflux, the mercury was returned from the condenser to the boiler by a small-bore mercury-sealed tube outside the vapor jacket. To keep the reaction chamber from being splashed with super-

heated mercury, and to obtain a more uniform flow of mercury vapor through the jacket, the boiler was set about 20 cm. from the latter and was heated electrically. A thin-walled thermometer well ending just above the reaction chamber and below the wide-bore outlet to the condenser was let into the top of the vapor jacket. The temperature determined from the pressure on the boiling mercury was found to agree with that measured by a platinum resistance thermometer at this point within 0.1° C. The thermometer was a small platinum-in-quartz instrument having a resistance of 25.00 ω at 0° C. It was calibrated at the freezing and boiling points of water and the boiling point of sulphur. It may be mentioned incidentally that this thermometer well has been found to be very convenient for the calibration of thermometers and thermocouples for other work. As before, the vapor jacket and boiler were heavily lagged with asbestos and all connections were glass-sealed.

Other changes in the apparatus are as follows. The two-way stopcock T_1 (3, Fig. 1) was replaced by a three-way tap, of which one way led to a tube where the reaction products could be condensed with carbon dioxide and ether for analysis. The lower part of this tube, which could be evacuated independently of T_1, was connected to the upper part by a 2-cm. mercury-sealed ground-glass joint and so could be removed for weighing. For greater ease of control during a run a stopcock was inserted between the tubes E and N. To make the manometer readings independent of the barometric pressure the open side (see diagram) of M_2 was connected to the high vacuum side of M_3. The pressure in the vacuum sides of the two manometers was kept below 10^{-4} mm. by occasionally evacuating through the large-bore mercury-sealed stopcock T_6.

In the new apparatus the distance between the mark C and the ring seal connecting B with the vapor jacket was made as much as 10 cm. in order to eliminate the possibility of the mercury surface at C being at a temperature lower than that of its surroundings. As before, the readings of M_2 and M_3 agreed well within 1 mm. when B was evacuated and the mercury surfaces at C and M_1 were level. Correction must of course be made for the smaller density of the mercury in the hot side of the manometer $C-M_1$.

It should be pointed out here that a further series of low pressure runs with ethylidene diacetate has been made in an apparatus in which the manometer was kept at the temperature of boiling xylene (vap. press. Hg = 1.7 mm.) instead of at that of the reaction chamber. The velocity data, which will be dealt with in detail elsewhere, are in excellent agreement with those already published (3) and so afford definite evidence that mercury vapor at pressures between 1.7 and 100 mm. is without influence on the rate of these reactions.

Preparation and Purification of the Esters

The butylidene diacetate was prepared by refluxing equimolecular quantities of c.p. butyraldehyde and acetic anhydride in the presence of about 0.1% H_2SO_4. When equilibrium (b.p. 140° C. at 760 mm. (4)) was reached the catalyst was neutralized with a calculated excess of fused sodium acetate and the mixture was roughly fractionated *in vacuo*. The last fraction (butylidene diacetate

plus acetic anhydride) was washed several times with water and finally with dilute baryta to remove the anhydride, dried over $CaCl_2$ and fractionated twice at atmospheric pressure. The middle fifth of the last fractionation was taken for these experiments. It boiled at 212° C., melted sharply at −6.5° C. and analyzed 0.5% acetic acid.

The ethylidene dipropionate was prepared by circulating dry acetylene through water-free propionic acid at 100° C. in the presence of about 1% of mercuric sulphate (prepared *in situ* from HgO and excess oleum) as catalyst. When the acetylene absorption had ceased, the catalyst was neutralized with an excess of fused sodium propionate and fractionated *in vacuo*. The last fraction was washed free of propionic acid with dilute baryta, dried with $CaCl_2$, and fractionated several times at atmospheric pressure. The middle third from the last fractionation was twice recrystallized and used for the decomposition experiments. It boiled at 191°, melted at about −19° C. and analyzed 2.2 per cent propionic acid. The acid in this ester, which appears to be more difficult to purify than the other two, was taken into account in the velocity measurements. Only a small yield of ethylidene dipropionate is obtained by this preparation, the greater part of the reacting propionic acid being converted to vinyl propionate.

Products of the Reactions

If the break up of butylidene diacetate and ethylidene dipropionate were strictly analogous to that of ethylidene diacetate, the reaction products would be butyraldehyde plus acetic anhydride, and acetaldehyde plus propionic anhydride respectively. A separate research (4) on the rate of formation of butylidene diacetate and on the equilibrium concentrations of aldehyde, anhydride and ester in the liquid state showed that below 200° C. the ester breaks up reversibly to butyraldehyde and acetic anhydride. Moreover in all the gaseous decompositions of this ester the pressure calculated by the ideal gas law from the weight of ester taken was equal (within about 2%) to half the final pressure obtained by back-extrapolation as already described (3). That is, the pressure just doubled during a run so that it is very improbable that products other than the aldehyde and anhydride were formed. In the case of this ester also the final pressure was practically stationary so that the extrapolations are more certain than those of the ethylidene diacetate runs, in which a very slow but continual pressure increase was observed. As with ethylidene diacetate, the value of the initial pressure calculated from the weight of the ester taken gave less satisfactory constants than were obtained by assuming the initial pressure to be equal to one-half the final pressure.

The agreement between the calculated and the observed pressures of ethylidene dipropionate was never as good as for the other two esters, presumably on account of the fact that the secondary reaction was somewhat faster and the extrapolation more uncertain than in the case of either ethylidene diacetate or butylidene diacetate. The fact also that the ethylidene dipropionate was not as pure as the other esters may have had something to do with this discrepancy. There seems, however, to be no reason for doubting that this ester also is decomposed quantitatively to an aldehyde and an anhydride.

In spite of many attempts to condense and analyze the reaction products no really satisfactory analysis was obtained. The quantitative condensation of such high boiling liquids appeared to be the main difficulty as the anhydride found was always less than that calculated on the basis of 100% ester decomposition. The discrepancy between the two values, however, was never large enough to suggest a decomposition other than that postulated. Since the back-extrapolated final pressure of every run was practically twice the back-extrapolated initial pressure and very nearly twice the calculated initial pressure, a 100% ester decomposition at all temperatures and pressures is assumed. Thus, as in the case of ethylidene diacetate, no correction is needed in the ordinary monomolecular equation for the velocity of the reverse reaction.

Results

Calculation of Velocity Constants

It was shown in the first paper of this series (3) that the monomolecular velocity constants of these decompositions are given by the expression

$$k = \frac{1}{t} 2.303 \log \frac{P_0}{2P_0 - P},$$

where P_0 is the pressure at the beginning of the reaction and P is the pressure at time t. The graphical method of evaluating k was also explained.

Instead of obtaining graphically an average velocity constant for all the runs at any one temperature, as was done in the case of ethylidene diacetate, the data of each butylidene diacetate and ethylidene dipropionate run were plotted separately on the same large scale as before, the best straight line was drawn through the points and the velocity constant was determined from its slope. These constants for all the butylidene diacetate and ethylidene dipropionate runs are given in column 6 of Table I, which includes also the temperatures at which the runs were made (column 2) the weights of ester used (column 3) the initial pressures, *i.e.*, one-half of the back-extrapolated final pressures (column 4) and the ratios P_0/weight of ester (column 5). It is not deemed necessary tc include here any graphs of $\log \frac{P_0}{2P_0 - P}$ against time as the curves for ethylidene diacetate (2, Fig. 2) are typical of these other esters. Practically all the butylidene diacetate runs gave perfectly straight lines until the reaction was at least 90% complete. The ethylidene dipropionate results were not as good as those for ethylidene diacetate and butylidene diacetate presumably on account of the above-mentioned uncertainty in determining the initial pressure. The velocity constants are not considered to be seriously in error, however, as their drift did not become appreciable until the reaction was 60-80% complete.

That the reaction velocity is independent of the initial pressure may be seen by comparing the values given in columns 4 and 6 of Table I for a set of runs at the same temperature. Thus in the butylidene diacetate runs 1, 2, 3, 4, 18 and 19 the initial pressures varied from 12 to 45.2 cm. without causing any appreciable trend in the velocity constant. In the ethylidene dipropionate

runs 1, 2, 3, 4, 5, 16, 17 and 18 the pressure varied from 8.3 to 55.5 cm. with the same result. There can thus be no doubt that the reactions are monomolecular.

The ratios in column 5 are included to show that the percentage decomposition is independent of pressure and to give an idea of the limits of error involved. No formal computation of errors has been attempted on account of the unknown extent of the deviation of such systems from the ideal gas laws, and because of the unknown (within a few per cent) purity of the difficultly analyzed esters. The consistent nature of the results obtained, however, indicate that the errors arising from such sources are relatively unimportant.

Homogeneity of the Reactions

In Runs 18 to 21 with butylidene diacetate and 4 to 9 with ethylidene dipropionate, the glass surface in contact with the decomposing ester was increased by loosely packing the reaction chamber with glass wool. As is evident from Table I no change in velocity occurred so that the reaction undoubtedly takes place homogeneously throughout the gas. These esters are similar to ethylidene diacetate in that several runs are sufficient to coat the interior of the reaction tube with a brown uneven film-like deposit, the growth or removal of which is without influence on the reaction rate. This film is considered to be the result of a side reaction, probably the polymerization of vinyl esters (3).

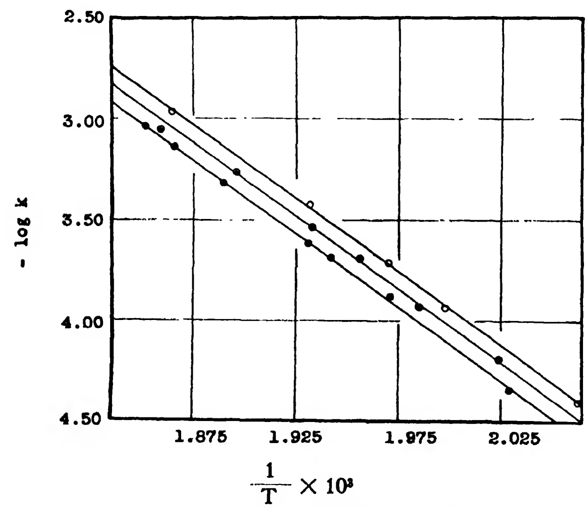


FIG. 1. *Graphs of -log k against* $\frac{1}{T}$. ●, *ethylidene diacetate;* ⊕, *ethylidene dipropionate;* ○, *butylidene diacetate.*

The Energy of Activation

In Table II is listed the reciprocal of the average absolute temperature (column 2) together with the negative logarithm of the average velocity constant (column 3) of all the ethylidene diacetate, butylidene diacetate and ethylidene dipropionate runs at each temperature. These values are plotted in Fig. 1 (ethylidene diacetate, ●; butylidene diacetate, ○; ethylidene dipropionate, ⊕), the straight lines of which were drawn parallel at a slope which would approximately fit all the points. This was done simply to emphasize, in the velocity-constant equations (column 4) and in the following discussion, the fact that the three activation energies are the same within experimental error, as well as to make the A's of the equation $\ln k = A - \frac{E}{RT}$ express the

observed differences of reaction rate. While such a procedure is admittedly arbitrary it is considered to be justified by the resulting simplification.

TABLE I

RESULTS OBTAINED IN RUNS WITH BUTYLIDENE DIACETATE AND ETHYLIDENE DIPROPIONATE

Run No.	$T°$, abs.	Weight of ester, gm.	P_0 cm. Hg.	Ratio $\frac{P_0}{\text{wt. of ester}}$	k (sec^{-1})
Butylidene diacetate					
1	517.8	0.2425	37.55	155	3.89×10^{-4}
2	517.9	0.1767	27.53	156	3.72×10^{-4}
3	517.8	0.1216	19.26	158	3.75×10^{-4}
4	517.7	0.2954	45.21	153	3.68×10^{-4}
5	535.9	0.1896	31.38	165	1.07×10^{-3}
6	536.0	0.1266	21.31	168	1.07×10^{-3}
7	536.1	0.0740	12.55	169	1.11×10^{-3}
8	552.0	0.1566	27.61	176	1.82×10^{-3}
9	507.6	0.2017	30.29	150	1.91×10^{-4}
10	507.6	0.2494	37.61	151	1.92×10^{-4}
11	507.6	0.1708	25.97	152	1.91×10^{-4}
12	507.6	0.0906	13.91	154	2.03×10^{-4}
13	484.4	0.1953	26.94	138	3.94×10^{-5}
14	485.5	0.3052	41.66	136	3.94×10^{-5}
15	484.6	0.1220	17.45	143	3.98×10^{-5}
16	500.4	0.1335	20.15	151	1.03×10^{-4}
17	500.4	0.1336	20.20	151	1.04×10^{-4}
18	517.8	0.1864	14.99	80.6	3.58×10^{-4}
19	517.8	0.1455	11.99	82.5	3.86×10^{-4}
20	500.5	0.1973	14.20	72.0	1.30×10^{-4}
21	500.6	0.1761	12.85	73.0	1.28×10^{-4}
Ethylidene dipropionate					
1	511.2	0.1348	19.95	148	2.02×10^{-4}
2	511.4	0.3121	44.40	142	2.19×10^{-4}
3	511.2	0.1749	25.56	146	2.11×10^{-4}
4	511.2	0.3690	26.23	71.2	1.95×10^{-4}
5	511.2	0.1170	8.28	70.8	1.97×10^{-4}
6	527.4	0.3746	27.79	74.3	5.44×10^{-4}
7	527.4	0.1393	10.08	72.3	5.50×10^{-4}
8	493.9	0.3581	24.04	67.1	$6.2\ \times10^{-5}$
9	494.0	0.1399	9.50	67.9	$7.0\ \times10^{-5}$
10	527.4	0.1690	12.25	72.1	5.57×10^{-4}
11	527.4	0.4150	31.75	76.5	5.35×10^{-4}
12	538.0	0.3966	29.02	73.3	9.10×10^{-4}
13	537.8	0.2091	15.40	73.8	8.70×10^{-4}
14	517.2	0.6483	45.46	70.1	2.72×10^{-4}
15	517.3	0.0867	6.25	72.1	3.10×10^{-4}
16	511.1	0.8019	55.49	69.1	1.87×10^{-4}
17	511.4	0.2218	15.47	69.9	2.19×10^{-4}
18	511.3	0.3346	23.28	69.6	1.98×10^{-4}
19	502.8	0.3933	26.87	68.5	1.15×10^{-4}
20	502.2	0.0904	6.30	69.6	1.24×10^{-4}
21	494.0	0.8245	53.0	64.4	6.40×10^{-5}

TABLE II

RECIPROCAL OF THE AVERAGE ABSOLUTE TEMPERATURE AND NEGATIVE LOGARITHM OF THE AVERAGE VELOCITY CONSTANT OF ALL RUNS WITH ETHYLIDENE DIACETATE, BUTYLIDENE DIACETATE AND ETHYLIDENE DIPROPIONATE AT EACH TEMPERATURE

Run No.	$\frac{1}{T} \times 10^3$	$-\log k$	$\ln k$
Ethylidene diacetate			
3, 4, 5, 6	1.942	3.688	
7, 8, 9	1.852	3.039	
10, 11	1.890	3.319	
12, 13, 14, 15	1.931	3.618	
16, 17, 18, 19, 20	1.866	3.140	
21, 22, 23	1.971	3.879	
24, 25	2.029	4.343	$\ln k = 23.74 - \frac{32,900}{RT}$
Butylidene diacetate			
1, 2, 3, 4, 18, 19	1.932	3.426	
5, 6, 7	1.865	2.967	
9, 10, 11, 12	1.970	3.712	
13, 14, 15	2.063	4.403	
16, 17, 20, 21	1.998	3.935	$\ln k = 23.96 - \frac{32,900}{RT}$
Ethylidene dipropionate			
1, 2, 3, 4, 5, 16, 17, 18	1.956	3.690	
6, 7, 10, 11	1.896	3.262	
8, 9, 21	2.024	4.185	
12, 13	1.859	3.051	
14, 15	1.933	3.536	
19, 20	1.990	3.925	$\ln k = 24.20 - \frac{32,900}{RT}$

Discussion

As is pointed out above, the majority of the monomolecular reactions hitherto investigated involve the more or less complete irreversible shattering of the whole molecule, so that it is not surprising that in general the activation energy decreases with increase of molecular complexity and instability. In the case of less drastic reactions that are strictly confined to a definite part of the molecule, as are these ester decompositions, it is to be expected that the energy of activation will be more independent of molecular structure, and that it might even appear as a constant characteristic of a series of compounds.

It is of course realized that conclusions drawn from the behavior of only three compounds of a homologous series must be regarded as purely tentative in their application to the series as a whole, and that future investigation may modify them considerably. The points which are raised by these experiments, however, are deemed to be sufficiently interesting to justify the present attempt to fit them in with what is already known about the break up of complicated molecules.

The assumptions made by Rice and Ramsperger (11) and by Kassel (5, 6), in the development of their theories II and III, seem to form the best foundation

for a discussion of the mechanism of these ester decompositions. Accordingly it is supposed that, as soon as the particular bond at which the molecule splits has accumulated an energy in excess of E, the energy of activation, reaction occurs; that a molecule is activated when the internal degrees of freedom which can contribute their energy to the reactive bond share in any distribution an energy in excess of E; and that the chance of the energy distribution resulting in reaction is proportional to the energy in excess of E possessed by the contributory degrees of freedom. In order to account for the Lindemann (9) time lag it must also be assumed that different energy distributions among these degrees of freedom succeed one another at a finite rate in the isolated molecule. Molecular activation is thus a result of collisional bimolecular processes governed by the ordinary Maxwellian distribution while "bond activation" is an affair of the isolated molecule being the result of an energy distribution over part of the molecular structure. As stated above "bond activation" and reaction are taken to be simultaneous events.

The fact that a structural alteration not affecting the particular part of the molecule involved in the reaction leaves the activation energy unchanged, favors the view that the latter is simply the minimum energy that the bond (or bonds) must acquire in order to react, as against the less definite but more rigorously derived identification of E as the difference between the average energy of the molecules which react and the average energy of all molecules*. It is hoped that the hypothesis outlined in the following paragraphs may be general enough to dispose of the essential differences between these two views of the physical meaning to be assigned to the energy term of the Arrhenius equation. It is advanced primarily in an attempt to explain the mechanism of simple structural changes taking place monomolecularly in complicated molecules.

The very common assumption, that a definite fraction only of the total number of internal degrees of freedom can contribute to the energy required at the weak point of a complicated molecule for decomposition to occur, appears to necessitate the further assumption that there are barriers within the molecule over which energy cannot easily pass (this has been suggested before (10)). That is, the molecule may be considered as being divided into sections between which, under conditions where the reaction velocity is measurable, there is no energy exchange and of which only the one containing the reactive bond can contribute to the energy of activation. That such ideas do not conflict with the experimental data on this point is evident when it is remembered that in practically all cases the number of degrees of freedom required to account for the observed reaction velocity is considerably less than the total number available. It is of course possible that the number found necessary to account for the reaction rate is a statistical quantity, sometimes including one particular degree of freedom and sometimes another. If this should be the case the accompanying speculations are meaningless. It is hoped that this point will be more definitely decided by experiments already referred to in which the

** For discussions of the meaning of the energy of activation see references 1, 5, 8, 10, 12 and 13.*

effect of various radicals, attached at different positions with respect to the breaking bonds, upon the velocity constant and activation energy is being investigated.

For the present it is assumed that as far as internal energy distribution is concerned the molecule is divided into different sections "insulated" from one another. A quantum oscillator having a frequency not commensurable with that of its neighbors would act as such an insulator (6). Energy could thus be furnished to the section containing the reactive bond only by direct collisions involving that part of the molecule, or by any collisions of sufficient violence to somehow break down the sectional insulation.

In a gas consisting of such sectionalized molecules, a Maxwellian energy distribution will exist among the molecular sections as well as among the molecules themselves. As it is the distribution among the reactive sections with which the Arrhenius equation is concerned, the energy of activation may be defined as *the difference between the average energy of the reactive sections which react and the average energy of all the reactive sections*. Such a definition would seem to combine the rigor of Tolman's derivation (12, 13) with the advantages inherent in the simpler view of the activation energy as a critical increment.

It is thus evident that the energy of the unreactive sections and, hence the total energy of the molecule, will have little or no effect upon the mechanism of activation or reaction. Indeed the nonreactive sections of the molecule might well have an even smaller influence on the velocity constant than if they were present as a separate inert gas which could activate and deactivate by ordinary collisions. The molecular diameter, velocity and mean free path, and hence the number and violence of the collisions, would of course depend upon the size and number of nonreactive sections in the molecule. It is well known, however, that the influence of such factors on reaction rate is overshadowed by the energy factor. A more important effect might well arise from a cushion or "bumper" action of the inert sections which would tend to diminish the number of effective collisions and would thus constitute at least part of the mechanism underlying the non-distribution of energy that must be assumed in order to account for the velocity of certain reactions (7).

In a series of first order reactions which have the same activation energy E, the relative decomposition velocities at any temperature will be proportional to the number of activated molecules, and also to the probability that the reactive bond will accumulate, from its particular section of the molecule, an energy in excess of E. Both the number of activated molecules and the probability that an activated molecule will react before the end of its mean free time are dependent upon the number (n) of degrees of freedom in what has been called above the reactive section. Thus if n is very small no appreciable fraction of the molecules will have energy greater than E in their reactive sections until the temperature has attained a very high value. If, on the other hand, n is large the same amount of energy will be available for the reactive bond at a proportionately lower temperature. An increase of n will thus increase the number of activated molecules but will tend to diminish the proportion that react, for the reason that there will now be a greater number

of possible energy distributions within the reactive section, and the chance of the occurrence of the one necessary for reaction will be reduced. It is to be expected, however, that this latter effect will be very small for small increases in n and, for the present, may be considered negligible in comparison with the increase in velocity due to the greater number of activated molecules.

The above hypotheses offer at least a qualitative interpretation of the ester decompositions reported in this paper. The addition of two $—CH_2—$ groups to ethylidene diacetate to form butylidene diacetate, and ethylidene dipropionate increases the reaction velocity without changing the energy of activation, and may thus be considered as simply adding to the number of degrees of freedom in the reactive section. That the magnitude of the velocity increase is dependent on the position of the added $—CH_2—$ groups is evident from a comparison of the reaction rates and structures of the three compounds. Butylidene diacetate has the greatest and ethylidene diacetate the smallest velocity at any temperature, with ethylidene dipropionate about half-way between. In butylidene diacetate both $—CH_2—$ groups are added to the aldehyde side of the ester while in ethylidene dipropionate both are on the anhydride side. The latter position would thus seem to be about half as effective as the former in adding to the degrees of freedom of the reactive section. Indeed, if the Arrhenius equation be written $\ln k = A - \frac{E}{RT}$ then, in a series of reactions having the same E, the numerical value of A may be taken as a relative measure of the number of degrees of freedom in the reactive section of the molecule. From Table II these values for ethylidene diacetate, ethylidene dipropionate and butylidene diacetate are respectively 23.74, 23.96 and 24.20. By studying the decompositions of other compounds of this series it is hoped to determine just what parts of the molecule can contribute energy to the reactive bonds and thus obtain information regarding the laws of intramolecular energy exchange. Further discussion of such matters will therefore be postponed until more experimental data are available.

References

1. CHRISTIANSEN, J. A. Proc. Cambridge Phil. Soc. 23: 438-449. 1926.
2. COFFIN, C. C. J. Am. Chem. Soc. 53: 3905-3906. 1931.
3. COFFIN, C. C. Can. J. Research, 5: 636-647. 1931.
4. COFFIN, C. C. and MILLER, PAULINE A. Trans. N.S. Inst. Sci. 18: 1-10. 1932.
5. KASSEL, L. S. J. Phys. Chem. 32: 225-242. 1928.
6. KASSEL, L. S. J. Phys. Chem. 32: 1065-1079. 1928.
7. KISTIAKOWSKY, G. B. and NELLES, M. Z. physik. Chem. Bodenstein-Festband, 369-378. 1931.
8. LEWIS, G. N. and SMITH, D. F. J. Am. Chem. Soc. 47: 1508-1520. 1925.
9. LINDEMANN, F. A. Trans. Faraday Soc. 17: 598-599. 1922.
10. RICE, O. K. Z. physik. Chem., Abt. B, 7: 226-233. 1930.
11. RICE, O. K. and RAMSPERGER, H. C. J. Am. Chem. Soc. 49: 1617-1629. 1927.
12. TOLMAN, R. C. J. Am. Chem. Soc. 47: 2652-2661. 1925.
13. TOLMAN, R. C. Statistical mechanics with applications to physics and chemistry. Chem. Cat. Co. 1927.

MEASUREMENT OF THE VISCOSITY OF GASES OVER A LARGE TEMPERATURE RANGE[1]

By B. P. Sutherland[2] and O. Maass[3]

Abstract

The work described is an investigation of the viscosity of air, hydrogen and carbon dioxide. The principle of damped oscillations was employed and an apparatus was built embodying many new features which make possible an accuracy greater than has hitherto been obtained. It was the attempt at the elimination of experimental error in the oscillating disk method which was the main feature of the investigation. At room temperatures where values in the literature are reliable excellent agreement with the best data has been obtained, but at lower temperatures where there is much divergence in published values the present results are of importance as giving more reliable and accurate data than hitherto available. For hydrogen and air the temperature range +20° to −200° C. was covered. In the case of carbon dioxide the range was limited by its properties to +20 to −95° C. As the temperature coefficient of the viscosity of gases is of particular interest from a theoretical point of view, as far as the kinetic theory is concerned, the data obtained may be considered important. Incidentally, Maxwell's law concerning the effect of pressure on viscosity was confirmed at the lowest temperature range hitherto investigated.

Introduction

The investigation of the viscosity of gases was really definitely begun by Graham about 84 years ago, and has been carried on by various workers with different types of apparatus up to the present. In spite of a vast amount of work and much data there is still a great deal of uncertainty and discordance, so that it becomes almost impossible to obtain sufficiently reliable data for satisfactory theoretical deductions. Even in the case of the viscosity of air at room temperature, upon which determination much labor has been expended, the value can be stated with certainty only to about 0.5%, and at lower temperatures there is so much divergence that it is impossible to deduce even a fairly good mean. The scarcity of reliable data at low temperatures is obvious from the survey of viscosity given in the International Critical Tables, where practically nothing is included on this property at low temperatures.

It was with a view to filling this gap to some extent and supplying data for theoretical deductions of molecular forces that the present work was undertaken. Unexpected difficulties and obstacles have hindered the covering of a wide field as yet, but it is believed that the results obtained are of a high order of accuracy and constitute a definite contribution to the subject. It is also expected that the increased knowledge of the best methods of attacking this problem will lead to further work on the viscosity of gases.

It would take too long to enumerate all the reasons for choosing the oscillating disk method for the determination of the viscosity of the gas. Suffice it

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to say that it was considered impossible to eliminate errors involved in the other methods when a large temperature range involving low temperatures was to be investigated, particularly when absolute values were to be determined.

The principle of damped oscillations was first used by O. E. Meyer (6, 7, 8). Tomlinson (12) and Hogg (2) further developed Meyer's method. Vogel (15) and Günther (1) evolved an apparatus which was the starting point of the one used by the present investigators.

Experimental Work

The marked improvements which have been made both in design of apparatus and experimental procedure will, it is hoped, be evident from the following description. The accompanying diagram (Fig. I) has been drawn fairly well to scale and will elucidate the description which follows.

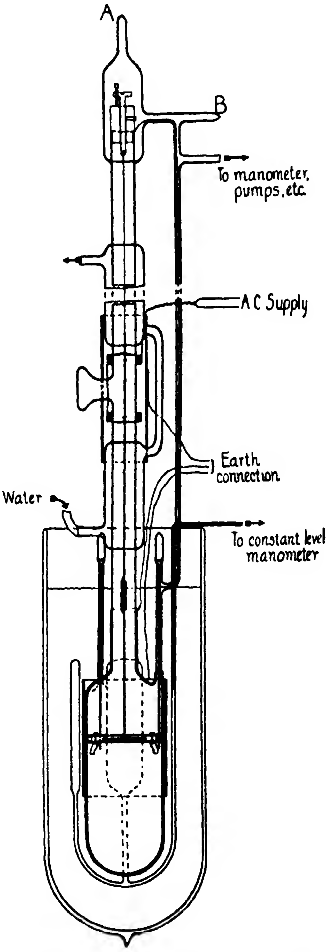

FIG. 1. *Apparatus for measuring the viscosity of gases over a large temperature range.*

The principle involved is the following. A circular disk is suspended in the gas so as to lie horizontally and is given a small torsional oscillation in its own plane. The rate at which the oscillations diminish is a measure of the viscosity of the surrounding gas.

Obviously a prime requisite is to have the place and means to hold the apparatus firmly and free from extraneous vibration. Such were obtained here in a room, partly below ground, in which the required supports were made by angle irons cemented into the wall. The apparatus itself was securely clamped to a heavy stand fitted with levelling screws. The stand was in turn clamped on to the wall bracket when accurately levelled. In this way it was found possible to keep the apparatus set in position as long as desired and unaffected by outside vibrations such as those due to work in other parts of the buildings.

The oscillating disk itself was made of silver, which is quite suitable for the purpose, and hung between two other disks of the same material, whereby the viscous drag was greatly increased. All three disks were 1 mm. thick. The swinging disk was 3 cm. in diameter and the fixed disks were about 4.5 cm. in diameter and were held apart by a spacing ring 3 mm. thick, the whole being fastened together with four small screws.

The amplitude of the oscillations was measured on a ground-glass scale by means of a beam of

light reflected from a small mirror mounted on a rod, which in turn was rigidly attached to the swinging disk. This rod was 1 mm. in diameter, about 25 cm. long, and a short section of it was made of ivory in order to hinder conduction of heat along it, as it was necessary to keep all of the upper part of the apparatus at constant temperature (25° C.) even though the lower part might be very cold.

The suspension wire carrying the rod and oscillating disks was of constantan, 0.001 inch in diameter and about 25 cm. long, and was attached to a hook fitting in an adjustable brass head so that it could be turned, raised or lowered and set in position. Though it was so fine, the wire was strong enough for the purpose and, with the particular dimensions here used, gave the system a period of oscillation of about 50 sec. Even after violent vibration due to accidents it was found to return to its original zero within very small limits.

The glass apparatus in which the whole was enclosed was of Pyrex and was made to order by The Fisher Scientific Company, its construction being clearly shown in the diagram. The jackets were for the circulation of water at 25° C. from a thermostat, to keep the suspension wire at constant temperature. Previously the water jackets had been of a removable type but the all-glass arrangement was an improvement. The construction at the head was so made that by blowing out the ends of the tubes *A* and *B* adjustments of the height or angular position of the disk could be made by means of a long screw-driver. Afterwards they could be sealed off again making the system air-tight.

Another respect in which this apparatus differed from previous ones was in the method of supporting the fixed disk. These were formerly fastened in place against the glass wall by means of litharge-glycerine cement. This was found to be unsatisfactory in that slight excess glycerine had an appreciable vapor pressure, and the cement itself was porous and very liable to absorb gases and then later give them out. In the present apparatus the disks were held in place with three rods whose heads rested in glass tubes as shown. These rods were threaded on the lower portions and fitted into holes in the edge of the disks. Accurate levelling could be done by merely turning the appropriate rods, their heads being slotted for the purpose. When the necessary adjustments had been made the ends of the tubes were sealed together ensuring the whole being air-tight.

Electrical connection was made between the disks by the wire shown which was entirely within a glass tube and connected with the head. The heat-insulating section of ivory was bridged by a very fine wire making electrical connection but having practically no heat effect.

Another feature of the glass work was the plane window opposite the mirror. This was formerly fixed on by means of sealing wax, but it was desired to avoid the use of all such materials if possible. Therefore it was fused on. An attempt was made to grind and polish the flattened end of a tube to form an optically true window but this was found very difficult to accomplish satisfactorily. Pyrex glass had to be used to make the join with the main apparatus. Finally some sheet Pyrex glass was supplied by the Corning Glass Works and it was found possible to fuse a disk of this to the end of a tube,

making a perfect join round the edges without heating the centre sufficiently to distort it. The over-all diameter of the piece made was about 3 cm. The central portion of this was entirely undistorted.

The original method of starting the oscillations was as follows: two small permanent magnets were astatically fixed to the rod of the oscillating disk and one of these was influenced with a permanent magnet from the outside. This was later replaced by a small solenoid through which a fixed current was passed when it was desired to produce a deflection and has again been substituted by two pairs of solenoids diametrically opposite one another. In this way a force is obtained on both magnets and this should closely approximate to a pure torque.

Further it was thought that the permanent magnets on the rod of the disk might themselves influence the results in two ways. First if they were not perfectly astatic there would be a tendency to set in one direction. Actually this was found to be the case and this direction was markedly affected by the presence of iron nearby. And secondly the force necessary to move the magnets through the earth's field would damp down the oscillations and so introduce an uncertain factor in the results. It is believed that these undesirable features have been almost entirely eliminated.

The two magnets themselves have been done away with and two small pieces of permalloy substituted. When starting the oscillations, the electrically produced field induces magnetism in these and by their tendency to align themselves with the field the oscillations are produced. On stopping the current the permalloy loses practically all its magnetism. Further the starting coils are all of the same size and connected in series, but the two pairs give opposite fields so that any residual magnetism should be neutralized. A source of supply of alternating current was obtained for starting, instead of direct current as heretofore, and this was gradually cut off each time so that the permalloy was demagnetized at the conclusion of each impulse. Care was taken to leave the coils on open circuit to avoid any induction effect due to the moving permalloy.

In order to prevent any effect from the earth's field or stray fields this section of the apparatus was encased in a shield of permalloy. As shown in the sketch, this extends considerably beyond the magnets and tests have shown it to be quite effective.

Electrostatic charges produced on the outside of the glass were found to produce violent motion of the disk inside, particularly when the apparatus was immersed in liquid air. This was overcome by the connection between the disks as mentioned above and by coating the outside of the glass with tin foil connected to ground. A permalloy shield around this section of the apparatus protected the moving disk from magnetic fields and was also grounded, as was the upper shield.

With these devices no appreciable effects from electrical or magnetic causes were observable under conditions which previously gave marked effects. Quite powerful permanent magnets are without any influence on the apparatus.

Accessory apparatus comprised the lamp and scale set. The lamp was of the type used in automobile headlights and gives a powerful concentrated beam. A new scale was made, 100 cm. long, and calibrated over the whole length. This was placed about nine feet from the mirror so the oscillations were magnified greatly. On such a long scale it is necessary to apply corrections for non-curvature. These have all been worked out and were used. This auxiliary apparatus was all firmly clamped on brackets similar to those on which the main apparatus was held.

From the viscosity apparatus a long zigzag tube, to avoid transmission of vibration, led to a McLeod gauge and mercury manometer. A Langmuir pump, Toepler pump, and containers of the various gases were connected when needed.

In setting up, the lower part of the apparatus was closed in an inverted position after the introduction of the disk. The small hook on the end of the suspension wire was inserted into the eye made for it in the brass head, and the apparatus was then set upright in its stand. The stand was then carefully levelled so that the swinging disk hung in the centre of the fixed disks. In this position it was rigidly clamped. Then the fixed disks were adjusted for horizontality by a cathetometer. Where these disks were supported with cement it was necessary to blow a small hole in the bottom of the apparatus so that adjustment might be made through this and the cement applied. This hole was afterwards sealed again. In the present form the adjustment was made by the long screws through the upper ends of the three tubes which were still open. Finally these tubes were sealed off.

The swinging disk was next adjusted so that it hung midway between the fixed disks and also so that the mirror threw the image approximately to the centre of the scale. When this was done the two tubes A and B were sealed off. It was necessary to have the scale perpendicular to the beam of light at the zero position. Adjustment of this was made from time to time as temperature and other effects produced slight shift of the zero.

A large Dewar flask of about 2½ litres capacity served as the bath for regulating the temperature of the gas. For temperatures above 0° C. water was used for this, and then ether down to about −100° C., cooling being done with solid carbon dioxide to about −70° C., and then with liquid air. Low boiling naphtha enabled lower temperatures to be obtained, the lowest actually used with this being about −123° C. Unfortunately this leaves rather a large gap before the next point which is that of liquid air (nearly all oxygen) about −183° C. Liquid nitrogen (93%) gave about 10° C. lower and by bubbling a rapid stream of hydrogen gas through this the temperature was reduced further to a minimum of −198.4° C. It is believed that under more favorable conditions of heat insulation this last method would be more effective and might be useful for conditions under which it was impossible to have the liquid under reduced pressure.

When needed, stirring of the bath was effected by a stream of dry air or hydrogen. Except in one or two cases the variation of temperature due to warming up during a run did not exceed 1° C., and at the higher temperatures

was much less than this. In all cases the variation was regular, so averaging gave accurate values.

Temperatures were measured by means of a hydrogen thermometer whose bulb was made in three parts as shown in the diagram. The reason for this shape was that it was desired to have a large volume distributed through the bath, and yet it had to be compact to fit into the Dewar vessel. The calibration was done by observations with a standard thermometer at room temperature and in liquid air, and showed that the ratio of volume of bulb to that of dead space was about 22. The temperature for the lower point was determined by ascertaining the proportion of oxygen in the gas coming off from the liquid air.

The corrections for variation in room temperature were calculated but were nearly always less than 0.1 mm. since the room temperature remained within a range of 2° C. most of the time. On the pressure scale, 0.2 mm. corresponds roughly to 0.1° C. of temperature, so temperature readings are correct to better than 0.2° C., the only uncertainty being when the temperature was changing relatively quickly.

The three gases used were carefully purified. Air was freed from moisture and carbon dioxide by passing through potassium hydroxide solution and sulphuric acid and then over phosphorus pentoxide.

Hydrogen was made in a Kipp generator from zinc and dilute sulphuric acid. The gas was passed through alkali hydroxide and sulphuric acid, dried over phosphorus pentoxide, and then passed through a bulb containing coconut charcoal at liquid air temperature.

Carbon dioxide was made from marble and dilute hydrochloric acid, and after purifying with carbonate solution and permanganate was dried and condensed to the solid by means of a carbon dioxide-ether mixture at reduced pressure. It was then slowly distilled over phosphorus pentoxide and condensed at $-78.5°$ C. under about $1\frac{1}{2}$ atm. It was stored under these conditions and before use was again passed over phosphorus pentoxide. A large portion was allowed to evaporate to ensure freedom from air or other non-condensable gases.

After some experience it was found advisable to have the apparatus at room temperature when a fresh gas was being introduced, in order as far as possible to prevent traces of the previous gas being held by adsorption. The apparatus was always pumped out, then filled with the new gas and allowed to stand for a while. It was then pumped out again and swept out several times before finally filling with the gas. The fact that samples of gas prepared at different times gave constant results is a proof not only of the reliability of the apparatus but also of the efficiency of the methods of purification.

The apparatus had first to be calibrated by using a gas whose viscosity was known. Air was chosen as being most suitable as its viscosity is fairly well determined at about 20° C., and in any case it is generally used as the standard of reference in other work. Accordingly the apparatus was pumped out and filled with air, free from carbon dioxide and carefully dried. After allowing time for it to come to the temperature of the bath the disk was set in oscillation and a number of consecutive readings were taken of the position of the image

on the scale at either end of its swings. Taking these in pairs and subtracting them obviously gives the amplitude of the oscillation, Ll, as read. A small correction for non-curvature is then applied giving the true amplitude, L. A sample set of readings is given in Table I to make this clearer. This was one of the standardization runs with air at 22.6° C.

TABLE I

STANDARDIZATION WITH AIR AT 22.6° C.

N	l	r	Ll	L	Log L	t
1	29.11	67.18	38.07	38.01	1.57990	0.00
2	31.12	65.23	34.11	34.06	1.53224	
3	32.94	63.52	30.58	30.54	1.48487	
4	34.52	61.97	27.45	27.42	1 43807	
5	36.00	60.60	24.60	24.58	1.39058	
6	37.31	59.37	22.06	22.04	1.34321	
7	38.49	58.22	19.73	19.71	1.29469	
8	39.51	57.25	17 74	17.73	1 24871	
9	40.46	56.35	15.89	15 88	1 20085	
10	41 32	55 58	14 26	14.25	1.15381	
11						831.0

In this table l and r are the readings to the left and right respectively. $Ll = r - l$, t is the time, taken by a stop watch, of a number of oscillations, in this case 10. This was measured from the time the image passed over the zero point of its swing in the direction left-to-right. The logarithmic decrement is now given by the difference between the values of log L for any two consecutive swings, and it is this function which gives at once a direct measure of the viscosity of the gas. Following Maxwell (4, 5) the set of readings as above was averaged by taking differences between the logarithms of the pairs: 1-10, 2-9, 3-8, 4-7, 5-6. If the logarithmic decrement is called λ then these differences will give respectively 9λ, 7λ, 5λ, 3λ, λ. These were now weighted by multiplying by their factors 9, 7, 5, 3 and 1 respectively, so that values of 81λ, 49λ, 25λ, 9λ and λ were obtained. These were added and divided by the total (165) giving the mean value of λ. The results obtained are shown in Table II.

TABLE II

$9\lambda = 0.42609$	$81\lambda = 3.83481$
$7\lambda = 0.33139$	$49\lambda = 2.31973$
$5\lambda = 0.23616$	$25\lambda = 1.18080$
$3\lambda = 0.14338$	$9\lambda = 0.43014$
$\lambda = 0.04737$	$\lambda = 0.04737$
	$165\lambda = 7.81285$
	whence $\lambda = 0.047351$

Two other such sets were obtained at the same time, the three being sufficiently close to allow of averaging.

In Table III, t is the time of one complete swing in seconds. The remarkably good agreement here shown is fairly conclusive proof that the apparatus is free from disturbing influences.

Before this value can be used for calculations it is necessary to determine certain corrections which have to be applied. In the first place the theory does

TABLE III

				Mean
λ	0.047351	0.047381	0.047387	0 047373
t, sec.	51.11	51 04	51.00	51.07
T, °C.	22.6	22 4	22 4	22.5

not give direct proportionality even for an ideal apparatus, but there is a variable factor which is expressed by Maxwell (4, 5) as a convergent series. The derivation of this would be out of place here but it may suffice to say that the first terms of the series were calculated for the case of air and are as follows, $1-0.00021+.00001-$etc. Obviously this can be put equal to 1 without affecting the accuracy of the results for present purposes.

The other corrections to be applied arise from the fact that other causes beside the drag of the gas on the disk will tend to damp down the oscillations. The rod attached to the disk and the mirror and magnets on it will all have a frictional force exerted on them by the gas, and as these parts are not at the temperature of the disk but at something close to 25° C. this force will not be constant in proportion to that on the disk. This force is supposed by Vogel (15) to be proportional to the square root of the viscosity but it is somewhat uncertain and not readily determined. In the present work the difficulty has been overcome by making these extra parts as small as possible so as to practically eliminate the effect. The mirror is about 2 by 3 mm. and the two magnets each about 2.5 by 0.8 mm. When it is remembered that the disk is 3 cm. in diameter, and the drag is proportional to the fourth power of the diameter it will be seen that the sum total of these is negligible, and as they are swinging freely in a wide tube the effect is again proportionately less. Experimentally these corrections can be determined only if the relative viscosity of two gases or of one gas at widely different temperatures is known. This method is used as a check and confirms the above conclusions.

It has already been explained how the possibility of magnetic and electrical effects causing a drag has been eliminated. Such little as may be left does not seem to be affected by any outside influences and so will be constant throughout.

Vibration will of course seriously affect the results. The substantial supports have reduced this to a minimum and if any unusual circumstances should cause such, it is readily noticeable. The period of oscillation in a transverse direction is so much smaller than that of torsional oscillation that such oscillation is visible in the movement of the image on the scale. Also it will make the results irregular so that if no such irregularity is found the apparatus may be presumed free from extraneous oscillations. In each case before beginning a set of readings the disk is brought to a practically steady position, then the swing of the desired magnitude is gradually built up. Before taking any readings a few complete swings are always allowed to take place to avoid any irregularities due to starting.

Thus there is left only one correction to be applied, namely, that for the internal friction of the wire suspension. This is of course independent of the gas being used and changes only with change in temperature of the wire. Such is avoided as far as possible by the jacket round all the upper part of the apparatus, which is thus kept close to 25° C. One difficulty arises here in that only a very slow stream of water can be passed through the jacket; otherwise a vibration is gradually built up. So, depending on the temperature of the lower part of the apparatus, the temperature of the incoming stream of water is varied to give a mean temperature of about 25° C. Thermometers in the water stream at the inlet and outlet show when this is obtained. If the elastic properties of the wire are affected by the temperature changes which do occur in it, the fact will be shown in changes in the period of oscillation. There is perhaps a slight decrease in the period when the lower part of the apparatus is very cold, but it is only a fraction of 1% and is not always observable, as the period is slightly lessened as the viscosity of the medium increases. Two cases in which the viscosity is about the same may be compared, namely, air at −185° and hydrogen at −100° C. The period was about 0.1% less in the former case.

The determination of the internal friction effect of the wire may be carried out directly by evacuating the system to a very low pressure, and observing the rate of decrease of the oscillations over a long period, such as one hour or more. The difficulty is to obtain the required high vacuum. As previously mentioned, a long connecting tube was used to avoid transmission of vibration and this greatly decreased the rate of diffusion of gas. Matters were improved by thoroughly sweeping out with hydrogen and then evacuating, but even so when the pump was running it was not at all certain that the pressure registered by the gauge was actually that in the apparatus. Indeed the following results prove that it was not.

After 15 hr. continuous pumping with a Langmuir pump backed by a Toepler pump the gauge showed a steady reading of 0.00003 mm. The mean of an extended series of readings gave a value of $\lambda = 0.001779$. After three days' pumping the gauge reading was still the same but $\lambda = 0.001460$, all being at room temperature. When the apparatus was cooled in liquid air the gauge reading fell to about 0.00002 mm., readings at these low pressures not being very certain, and the logarithmic decrement fell to 0.001234. The apparatus was then allowed to warm up again and after a further 12 hr. pumping the previous results at room temperature were closely repeated, namely $\lambda = 0.001466$ and $P = 0.00003$ mm. Unfortunately before further readings were taken, the Toepler pump broke down after nearly five days continuous running.

However, the pump was repaired and the apparatus once more filled with hydrogen and cooled in liquid air. Now about three hours' pumping brought the gauge reading down to 0.00002 mm. and $\lambda = 0.001226$. This is considered very fair agreement with that previously obtained, bearing in mind the smallness of the factor and the length of time required to get accurate results. At liquid air temperature there will be practically no gas given off from the metal, glass or cement so that the value obtained ($\lambda = 0.00123$) is probably a true

value of the internal friction of the wire. At room temperature this friction may be somewhat larger, but the increased value of λ is believed to be mainly due to vapor given off in the apparatus, of which the cement is the most probable source. As an approximation the effect was assumed proportional to the temperature but this approximation is not a serious one as will be seen when the method of applying the correction is shown. In fact whether the correction is put in as 0.0014 or 0.0012 will make a difference in the result of about 0.2% in the case of carbon dioxide at higher temperatures. For other cases the effect is smaller or larger according to whether the viscosity being determined is closer to or further from that of air at room temperature.

In the later form of apparatus there were no cements of any kind so that the only hindrance to obtaining high vacuum was the evolution of gas from the metal and glass. The procedure in this case was the same as before, pumping out being done after filling with hydrogen. Pumping for 36 hr. failed to give a constant value of the logarithmic decrement at room temperature so the apparatus was cooled in liquid air as before. This would effectually stop evolution of gas from the solid surfaces and ensure that the gas pressure was really low. Several hours further pumping brought the decrement to a steady value within experimental error. Three sets of readings spread over about 1½ hr. gave the results 0.000758, 0.000703, 0.000731, which are in good agreement and give a mean of 0.000731. This value was accepted as reliable for the present work and is used in the calculation of all results obtained with the apparatus in the later form. In view of the fact that there were no materials present which would exert an appreciable vapor pressure at room temperature the one value of the correction was used for the whole range. The extension to the higher temperatures is justifiable as the results prove.

It will be noticed that the correction factor is only about 60% of that obtained previously with wire of the same size and kind. This reduction was obtained by annealing the wire *in situ* following the experience of Tomlinson (12) with other wire suspensions. The annealing was done at a relatively low temperature by passing steam through the jacket for several hours, but it seems to have had a good effect in the desired direction.

The way in which this single correction is applied is easily understood. The sample run given above was made with the later form of apparatus so the correction which applies is $x = 0.000731$. Now λ represents the effect of the gas and the wire so that due to the gas alone is $0.047373 - 0.000731$ or 0.046642. This is the decrement for one complete oscillation of time t, so that per second is $\frac{\lambda - x}{t}$. If the viscosity of the gas is η, then $\eta = \frac{\lambda - x}{Ct}$ where C is the constant of the apparatus. The run in this case was made with air and was for the purpose of determining the constant C. As previously mentioned, Millikan's values for the viscosity of air are used for this work in order that results should be comparable with those given in the International Critical Tables. At the temperature concerned (22.5° C.) air has a viscosity of 1820.2×10^{-7} whence the constant is calculated and comes out as $C = 5.0176$. Of course any changes in the apparatus change the value of the constant, and during some of the runs

with the later form the constant was inadvertently changed by the repeated cooling and warming. This worked the disks up a little at one side, so the adjusting tubes had to be opened and the screws fastened in place with DeKhotinsky cement. Fortunately this did not occur until after the wire correction was determined so that this remained unchanged. In any future work this difficulty can easily be overcome by altering the springs so that they do not hold the disks up, or by fastening a heavier piece to the disks so that they are always holding the screws down in their seats. After this experience of changing constant a check run at room temperature was made after every few runs to detect any variation. Such was not found to occur and so the calculation of all intermediate values can be made with confidence.

As an additional check on the constant and corrections of the apparatus, a series of experiments was carried out with hydrogen at room temperature. The viscosity of this gas has also been determined by Millikan's students with the same apparatus as used for air, so it may be taken as a reliable comparative value. Perfect agreement with this result was obtained with the present apparatus, which was gratifying as confirming the previous calibration as well as substantiating the value of the viscosity of hydrogen to some extent. For any future work of a similar kind it will be useful to have these accurate comparative figures on air and hydrogen. These gases form excellent calibrating materials since they are readily obtainable in a pure state and have such widely different viscosities. If there were any other correction which should be accounted for it would have been evident in the comparison with the two gases, but apparently those detailed above are all that are needed for the purpose.

Having completed the calibration of the apparatus the viscosities of the various gases were determined at the required temperature. Precisely as in the sample run, λ was determined from a series of readings. Knowing C and x, η is calculated from the formula.

At temperatures below the boiling point of the gas, reduced pressures must be used. It is well known that, over a wide range, the viscosity is independent of the pressure, so the results obtained are comparable with those in which atmospheric pressure was used. The validity of this conclusion, even at low temperatures, was confirmed by experiments over a wide pressure range. This apparatus was found to give the same values from 76 cm. down to about 2 cm. At 1 cm. pressure a slight falling-off is generally noticed. In view of this no experiments were carried out at less than 3 cm., which is safely over the limit required.

Results

The first part of the work was done with the apparatus in which the disks were held to the wall with cement. The results are shown in Table IV.

It was thought that the values in the literature for the viscosity of air at room temperature and at $-78.5°$ C. might be sufficiently dependable to allow of the calculation of the constant and correction of the apparatus. The equation as already given is $\eta = \frac{\lambda - x}{Ct}$, so by substituting the two sets of values

TABLE IV

λ	t, sec.	T, °C.	λ	t, sec.	T, °C.
Air			Carbon dioxide		
.039487	47.56	12.6	.033026	47.54	21.8
.039480	47.52	12.7	.030658	47.54	1.3
.039519	47.56	12.8	.028442	47.52	−19.4
.028807	47.48	−78.5	.026139	47.49	−40.2
.028804	47.48	−78.5	.024116	47.50	−60.0
.028852	47.48	−78.5	.022174	47.48	−78.2
			.020535	47.50	−97.8

of η, λ, and t, one can solve for C and x. The value of η at the higher temperature was obtained by interpolation from the data in the International Critical Tables, namely, $\eta=1772.3\times10^{-7}$ at 12.7° C. At −78.5° C. results by Vogel (15) and Schmitt (11) are available and give a mean of 1304×10^{-7} after correcting to refer to the same room temperature standard. With these figures, x comes out as an appreciable negative quantity which is impossible. Evidently the trouble lies with the assumed viscosity at −78.5° C. The difficulty was overcome by determining x directly by experiments at low pressure. These have already been explained and the results given. The values applying here are those varying from 0.00146 at 20° C. and 0.00123 at −183° C. Therefore the calculated constant for these experiments comes to be $C=4.5144$. Using this, the viscosities are as shown in Table V.

TABLE V

VISCOSITIES OF AIR AND CARBON DIOXIDE

Gas	Temp, °C.	$\lambda-x$	$\eta\times10^7$
Air	−78.5	.024747	1281.7
Carbon dioxide	21.8	.031565	1470.8
Carbon dioxide	1.3	.029212	1361.1
Carbon dioxide	−19.4	.027029	1259.9
Carbon dioxide	−40.2	.024750	1154.5
Carbon dioxide	−60.0	.022748	1060.8
Carbon dioxide	−78.2	.020825	971.6
Carbon dioxide	−97.8	.019208	895.8

With the later form of apparatus results were obtained with air and hydrogen at room temperature as well as the direct determination of the wire correction x. However, during the next experiments which were with carbon dioxide, slight movements of the disks occurred so that these were valueless. Obviously the wire correction is not affected by such changes so it applies throughout as 0.000731, but the constant C had to be redetermined for further work. The results for air on this occasion are those given earlier leading to $C=5.0176$, and the only calculation in which this applies is that for hydrogen at 20.8° C. In this we have:

$\lambda=0.023192$, $\lambda-x=0.022461$, $t=51.08$, whence

$\eta=876.4\times10^{-7}$. This agrees well with Millikan's (9,10) value which by extrapolation to this temperature is about 877.6×10^{-7}.

As previously explained, the change in the constant of the apparatus was due to changes in temperature. That this is so is proved by the fact that, when

experiments immediately following one another were made on air and carbon dioxide at about the same temperature, good agreement with previous results was obtained as:

Air at 20.8° C., $\lambda = 0.049678$, $t = 51.10$, gives $C = 5.2861$.

Carbon dioxide at 21.8° C., $\lambda = 0.040477$, $t = 51.16$, with the above C, $\eta = 1469.7 \times 10^{-7}$. This is plotted on the curve with other values and falls well into line. When the apparatus had been cooled in the interval, however, divergences of 5% or more were found.

TABLE VI

STANDARDIZATION OF APPARATUS WITH CARBON DIOXIDE (CONSTANT $C = 4.5559$)

Temp., °C.	λ	t	$\eta \times 10^7$
0.0	0.032307	51.09	1356.6
−65.6	0.024867	51.03	1035.6

After locating and remedying the trouble, the apparatus was again standardized, this time using carbon dioxide at room temperature. After experiments at lower temperatures, another standardization at room temperature was carried out. This agreed with that previously obtained within 0.1%. The results so obtained are shown in Table VI.

Some experiments were next carried out in liquid air and because, as was later found out, part of the tin foil coating had come off the apparatus and the ground connection was broken, no satisfactory readings could be obtained. Even when the bath full of liquid air was brought up round the apparatus with the greatest care, most violent movement of the swinging disk was caused, throwing the image completely off the scale. After allowing a long time for settling, the spot still had a movement of several centimetres and great discordance in results was obtained. Addition of more liquid air to the bath caused further violent movement and all the time the zero position kept changing over a range of several centimetres. When the liquid air was removed and the apparatus allowed to warm up to room temperature, further violent oscillations were observed but finally the image settled to a steady position on the scale not more than 1 mm. from the previous position of rest at room temperature. At this temperature, with the apparatus filled with air, a set of experiments was carried out giving results that checked closely with the preceding ones on carbon dioxide, showing that the procedure just described had not affected the working of the apparatus at room temperature.

However, in order that it might be possible to do the low temperature work, the apparatus was thoroughly cleaned off and the whole of the lower part covered with tin foil which was earthed. Unfortunately in this work it was impossible to avoid slight disturbance to the apparatus so it was necessary to reset it and to make a further standardization. This was done with air at room temperature as usual and gave the result $C = 4.5900$. This treatment was found to be entirely effective in preventing the disturbances before observed with liquid air, thus showing fairly conclusively that electrostatic charges were responsible.

All the rest of the experiments described herein were completed without altering the constants of the apparatus again. This is proved by the fact that experiments with hydrogen at room temperature, and also with carbon dioxide at various temperatures, gave results agreeing with those previously determined. The results are collected and arranged in Table VII. Generally the value of λ given is the mean of two distinct sets of readings each involving the measurement of at least ten complete oscillations. In a few cases it is the mean of three or more such sets. As previously mentioned, slight temperature changes occur during some of the runs so of course the value of λ will change also. This was always small, however, and the results may be averaged, since they are practically proportional to the temperature. Experimental errors never gave rise to variations exceeding 0.4% and usually were considerably less. The values of η have a small correction applied to them to allow for the contraction of the disks at the lower temperatures. This is proportional to the temperature to a sufficiently close approximation and is of the order of 0.1% at $-70°$ C.

TABLE VII

RESULTS OBTAINED WITH AIR, HYDROGEN AND CARBON DIOXIDE

Gas	T, °C.	t	P	λ	$\lambda - x$	$\eta \times 10^7$
Air	20.8	51.09	1 atm.	0.043214	0.042483	1811.6
Air	0.0	51.07	1 atm.	.040776	.040045	1708.3
Air	−31.6	51.08	1 atm.	.036791	.036060	1539.2
Air	−69.4	51.04	1 atm.	.031908	.031177	1332.8
Air	−104.0	51.01	1 atm.	.027124	.026393	1129.5
Air	−183.1	50.85	1 atm.	.015314	.014583	626.9
Air	−194.2	50.92	8 cm.	.013562	.012831	551.1
Hydrogen	20.7	51.11	1 atm.	.021291	.020560	876.4
Hydrogen	0.0	51.07	1 atm.	.020307	.019576	835.1
Hydrogen	−31.6	51.08	1 atm.	.018695	.017964	766.9
Hydrogen	−62.6	51.02	1 atm.	.017108	.016377	700.6
Hydrogen	−97.5	50.97	1 atm.	.015075	.014344	615.2
Hydrogen	112.6	50.98	1 atm.	.014205	.013474	576.9
Hydrogen	−113.5	50.98	1 atm.	.014074	.013343	571.5
Hydrogen	−123.7	50.95	1 atm.	.013507	.012776	547.5
Hydrogen	−183.4	50.91	1 atm.	.009776	.009045	388.4
Hydrogen	−195.2	50.92	1 atm.	.008775	.008044	345.4
Hydrogen	−198.4	50.93	1 atm.	.008558	.007827	336.0
Carbon dioxide	−35.0	51.04	1 atm.	.028485	.027754	1184.7
Carbon dioxide	−73.7	50.99	1 atm.	.024191	.023460	1002.4
Carbon dioxide	−95.4	50.98	3 cm.	.021931	.021200	906.0

The first of these values for air is the standard from which the constant is calculated. It is $C = 4.5900$. From this all the other values were determined.

The relation between viscosity and temperature for each of the three gases is clearly shown by the accompanying curves (Fig. 2). It will be seen that at the higher temperatures the relation is nearly a straight-line function but curvature is shown as the temperature falls. It is interesting to note that in the case of carbon dioxide the slope decreases, whereas the reverse is true in the cases of air and hydrogen. In comparing the curves it should be remem-

bered that the values for carbon dioxide are all below the critical temperature (31.1° C.), and for hydrogen they are all above (−234.5° C.), while air is intermediate, its critical temperature being about −140° C.

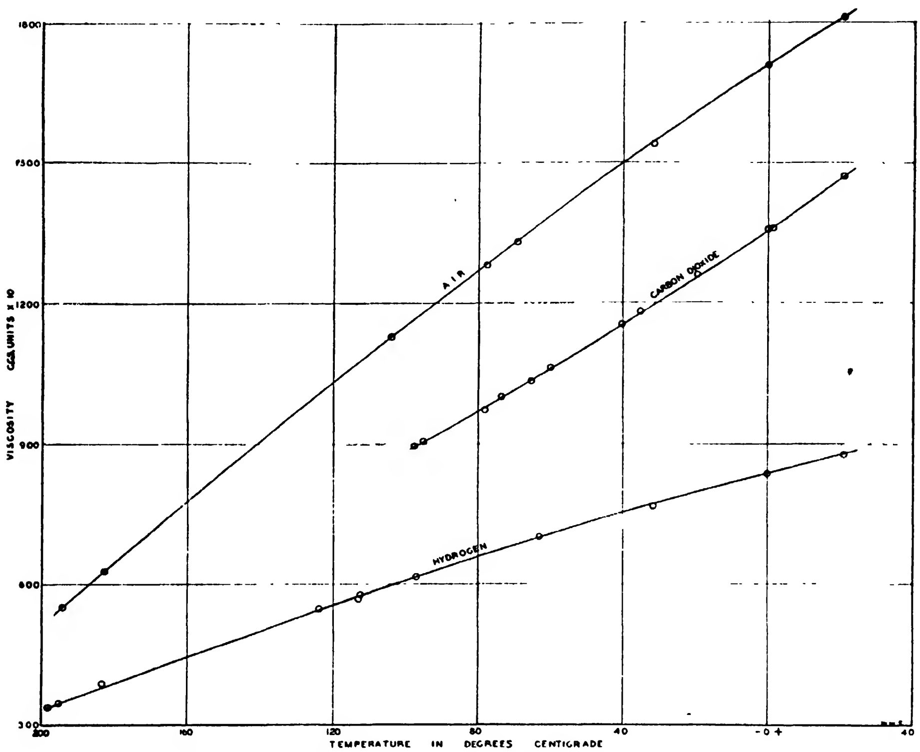

FIG. 2. *Variation of viscosity with the temperature.*

The accepted values for carbon dioxide given in the International Critical Tables are too high at the lower temperatures. At room temperature there is good agreement with the values of Vogel (15), Van Dyke (14), Ishida (3), and Trautz and Kurz (13).

For air the only points of comparison available from the International Critical Tables are at and above 0° C. and as the values at higher temperatures were used for calibration the one point left to compare is at 0° C. Here agreement is to within 0.1%, which is excellent.

In the case of hydrogen the values in the I.C.T. are more conflicting. However it is believed that the value obtained by Millikan's students is at least as reliable as any other and with this the present results are in agreement to about 0.1%. At the lowest temperatures again, close agreement with the values in the I.C.T. is shown although at intermediate points the present results tend to be somewhat smaller than those given previously. However, this is by no means a proof of inaccuracy, as the literature will also give results in conformity with the present ones if care were taken to select them from the mass of data.

Actual figures on one other point should be given, *i.e.*, on the relation between viscosity and pressure at low temperatures. It was found that in each case the logarithmic decrement began to fall off at about 1 cm. as previously mentioned. This was doubtless due to the "slip" effect, the viscosity itself being constant. In any event viscosity as measured by this apparatus is constant down to 2 cm. The results are given in Table VIII.

So from this point of view these gases behave the same way at low temperatures as they do at higher ones.

TABLE VIII

RELATION BETWEEN VISCOSITY AND PRESSURE AT LOW TEMPERATURES

Gas	Temp., °C.	Pressure	λ
Air	−183.1	1 atm.	.015314
Air	−183.0	30 mm.	.015363
Air	−183.0	20 mm.	.015348
Air	−183.0	10 mm.	.015403
Hydrogen	−183.0	1 atm.	.009776
Hydrogen	−183.0	10 mm.	.009753
Carbon dioxide	− 78.5	400 mm.	.025095
Carbon dioxide	−78.5	10 mm.	.024917

In conclusion it may be stated that, for the work as a whole, it is claimed that with one and the same apparatus a complete set of concordant results has been obtained covering a wide temperature range, so that it may be safely concluded that the shape of the viscosity-temperature curves is that shown in the diagram. The analysis of these results from the point of view of information of theoretical interest will be considered in a subsequent paper.

The claim is made that the data given are of a greater accuracy than any available, except those at room temperature, and for this reason the improvement in the apparatus and the detail of the experimental procedure has been given very fully in order to substantiate this claim.

An extensive programme involving the investigation of other gases is now under way.

References

1. GÜNTHER, P. Z. physik. Chem. 110: 626-636. 1924.
2. HOGG, J. L. Proc. Am. Acad. Arts Sci. 42: 115-146. 1906.
3. ISHIDA, Y. Phys. Rev. 21: 550-563. 1923.
4. MAXWELL, J. C. Phil. Trans. 156: 249-268. 1866.
5. MAXWELL, J. C. Collected works, v.2. 1-78 Cambridge Univ. Press. 1890.
6. MEYER, O. E. Kinetic theory of gases. 171-246. Longmans. 1899.
7. MEYER, O. E. Crelles Journal, 59: 229-230. 1861.
8. MEYER, O. E. Pogg. Ann. 148: 1-203. 1873.
9. MILLIKAN, R. A. Ann. Physik, (4), 41: 759-766. 1913.
10. MILLIKAN, R. A. Phys. Rev. 2: 109-143. 1913.
11. SCHMITT, K. Ann. Physik, (4), 30: 393-410. 1909.
12. TOMLINSON, H. Proc. Roy. Soc. Lond. 40: 40-42. 1886.
13. TRAUTZ, M. and KURZ, F. Ann. Physik, (5), 9: 981-1003. 1931.
14. VAN DYKE, K. S. Phys. Rev. 21: 250-265. 1921.
15. VOGEL, H. Ann. Physik, (4), 43: 1235-1272. 1914.

A PHOTO-ELECTRIC CELL CIRCUIT[1]

By G. A. Woonton[2] and R. G. Elson[3]

Abstract

In this paper it is shown that a vacuum tube circuit of special design is capable of linear response to the varying conductivity of a photo-electric cell. A method is suggested whereby light intensities of widely different magnitudes can be compared. The circuit is shown to be extremely stable.

Fig. 1 is the diagrammatic representation of a circuit developed by Hull (4) for use where fluctuations in light intensity are to be reproduced electrically by means of a photo-electric cell. For the present purpose it has been re-adapted to give quantitative measurement of the amount of ultra-violet light in sunshine and skyshine, and to determine the ultra-violet reflecting and transmitting properties of various materials. The same circuit may be used as a densitometer for the measurement of film densities or, with a very slight change, as a resistance meter for the measurement of the variation in the resistance of crystals and salts where the resistances involved are of a large order of magnitude.

Circuit Theory

It is obviously impossible to calibrate the circuit by illuminating the cell and measuring the input and output currents over a range of light intensities. The investigation of the nature of the amplification can be made only on a theoretical basis.

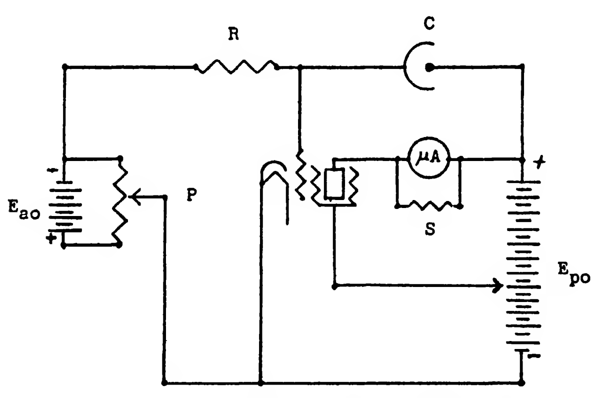

Fig. 1. *The original circuit. μA, Micro-ammeter; C, photo-electric cell; E_{ao}, "A" grid bias battery; E_{po}, plate battery; P, potential divider; R, high resistance; S, shunt; tube, UY-224.*

Referring to Fig. 1—if the resistance of the photo-electric cell is altered by a change in the energy falling on the cell, the current flowing in the outer circuit C, R, E_{ao}, E_{po} is altered and the potential drop across R changes. An increase in current flowing through R, because of the polarity of the batteries, must be such that the end of R next to the control grid becomes more positive with respect to the other end. E_{ao}, the potential due to the biasing battery, gives the control grid a constant negative bias with respect to the cathode. The effective bias of the control

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grid must therefore be made up of two parts—a constant bias E_{ao} and a varying bias in the opposite direction due to the current flowing through the high resistance R. The current flowing in the output circuit, according to the usual theory, is a function of this bias.

For the purpose of this analysis it should be noted that it is necessary to define the resistance of the photo-electric cell C, as the reciprocal of the conductivity of the cell at constant anode voltage but with varying light flux. This is contrary to usual practice. C in this case is a variable even though the ratio $\frac{\text{anode voltage}}{\text{anode current}}$ is a constant for constant light flux.

The type of mathematical analysis used in determining the form of the function is that developed by Carson (2), Llewellyn (5) and Brainerd (1).

Let E represent a potential difference and I a current. Let the subscripts p, a and b refer to the plate, control-grid and screen-grid circuits respectively. Further, let the subscript o indicate a steady condition and the lower case letters e or i indicate a variation around such a steady value.

We may write for the various circuits

$$\begin{aligned} E_p &= E_{po}+e_p & I_p &= I_{po}+i_p \\ E_a &= E_{ao}+e_a & I_a &= I_{ao}+i_a \\ E_b &= E_{bo}+e_b & I_b &= I_{bo}+i_b \end{aligned} \tag{1}$$

The following functional relations are quite general:—

$$\begin{aligned} I_p &= I_p(E_a, E_b, E_p) \\ I_a &= I_a(E_a, E_b, E_p) \end{aligned} \tag{2}$$

If $e_a=e_b=e_p=0$, the plate current must be in its normal steady state and $i_p=0$. Then

$$I_{po}=I_p(E_{ao}, E_{bo}, E_{po})$$

From Equation (1)

$$i_p=I_p-I_{po}$$

therefore

$$i_p=I_p(E_a, E_b, E_p)-I_p(E_{ao}, E_{bo}, E_{po})$$

and similarly

$$\begin{aligned} i_a &= I_a(E_a, E_b, E_p)-I_a(E_{ao}, E_{bo}, E_{po}) \\ i_b &= I_b(E_a, E_b, E_p)-I_b(E_{ao}, E_{bo}, E_{po}) \end{aligned} \tag{3}$$

Each of these relations may be developed into a Taylor's series evaluated in the neighborhood of the steady values.

$$i_p = \frac{\partial I_p}{\partial E_a}e_a+\frac{\partial I_p}{\partial E_b}e_b+\frac{\partial I_p}{\partial E_p}e_p+\tfrac{1}{2}\frac{\partial^2 I_p}{\partial E_a^2}e_a^2+\tfrac{1}{2}\frac{\partial^2 I_p}{\partial E_b^2}e_b^2+\ldots, \tag{4}$$

which may be written for convenience,

$$i_p=P_1e_a+P_2e_b+P_3e_p+P_4e_a^2+P_5e_b^2+P_6e_p^2+\text{etc.}, \tag{5}$$

and similarly

$$i_a=A_1e_a+A_2e_b+A_3e_p+A_4e_a^2+A_5e_b^2+\text{etc.}$$

Since the resistance of the output meter and its accompanying shunt is insignificant E_p must be constant. Therefore $E_p=E_{po}$ and $e_p=0$; by similar reasoning

$$e_b=0$$

whence

$$i_p = P_1 e_a + P_4 e_a^2 + \text{etc.},$$
$$i_a = A_1 e_a + A_4 e_a^2 + \text{etc.} \tag{6}$$

In the a grid circuit R may be thought of as a source of e.m.f., due to the drop in potential across it when a current flows in the circuit C, R, E_{ao}, E_{po}. If the potential difference due to this cause be defined as e, we have

$$e = \frac{E_{ao}+E_{po}}{R+C} R = \frac{VR}{R+C}, \tag{7}$$

if $P << R$, where P is resistance of potential divider, C is the resistance of the photo-electric cell, and V is the effective potential applied to the cell circuit.

If the value of C decreases, the grid becomes more positive relative to the cathode. The balance between secondary and primary emission from and to the grid is disturbed by the change in potential, and I_a varies slightly. The variation i_a in the grid current causes a drop in potential across R in the opposite direction to that caused by the current in the outer circuit; the value of the change in the potential drop must be $i_a R$. The net change in the potential of the grid with respect to the cathode may be written

$$e_a = e - i_a R \tag{8}$$

We may express i_a as a formal power series in terms of e

$$i_a = b_1 e + b_2 e^2 + \ldots\ldots\ldots\ldots +, \tag{9}$$

where the b's are undetermined coefficients.
Substituting in Equation 8

$$e_a = e - (b_1 e + b_2 e^2 + \ldots\ldots\ldots\ldots)R$$
$$= (1 - b_1 R)e - b_2 R e^2 + \ldots\ldots\ldots\ldots + \tag{10}$$

Substituting (9) and (10) in (6)

$$b_1 e + b_2 e^2 + \ldots\ldots\ldots + = A_1[(1-b_1R)e - b_2Re^2 + \ldots\ldots +]$$
$$+ A_4[(1-b_1R)e - b_2Re^2 + \ldots\ldots + \ldots]^2 \tag{11}$$

From which b_1 and b_2 may be evaluated by equating like powers of e,

$$b_1 = \frac{A_1}{1+A_1R} \qquad b_2 = \frac{A_4}{(1+A_1R)^3}. \tag{12}$$

Therefore

$$e_a = (1 - \frac{A_1R}{1+A_1R})\, e - \frac{A_4Re^2}{(1+A_1R)^3} + \ldots\ldots\ldots + \ldots\ldots$$

$$= \frac{e}{(1+A_1R)^3}[(1+A_1R)^2 - A_4Re + \ldots\ldots +]. \tag{13}$$

But according to Equation (6)

$$i_p = P_1 e_a + P_4 e_a^2 + \ldots\ldots\ldots\ldots\ldots\ldots +.$$

Therefore we may write

$$i_p = \frac{P_1 e}{(1+A_1R)^3}[(1+A_1R)^2 - A_4Re + \ldots\ldots + \ldots\ldots +]$$

$$+ \frac{P_4 e^2}{(1+A_1R)^6}[(1+A_1R)^2 - A_4Re + \ldots\ldots]^2 + \ldots\ldots\ldots\ldots + \tag{14}$$

By definition

$$\mu_a = \frac{\dfrac{\partial I_p}{\partial E_a}}{\dfrac{\partial I_p}{\partial E_p}} \qquad \mu_b = \frac{\dfrac{\partial I_p}{\partial E_b}}{\dfrac{\partial I_p}{\partial E_p}}$$

$$\frac{1}{r_p} = \frac{\partial I_p}{\partial E_p} \qquad g_a = \frac{\mu_a}{r_p} = \frac{\partial I_p}{\partial E_a} \tag{15}$$

$$g_b = \frac{\mu_b}{r_p} = \frac{\partial I_p}{\partial E_b}$$

$$\frac{1}{r_{ga}} = \frac{\partial I_a}{\partial E_a} \qquad \frac{1}{r_{gb}} = \frac{\partial I_p}{\partial E_b}$$

The P's and A's may be expressed in terms of these definitions

$$P_1 = \frac{\partial I_p}{\partial E_a} = g_a \qquad A_1 = \frac{\partial I_a}{\partial E_a} = \frac{1}{r_{ga}} \tag{16}$$

$$P_4 = \tfrac{1}{2}\frac{\partial^2 I_p}{\partial E_a^2} = \tfrac{1}{2}\frac{\partial g_a}{\partial E_a} \qquad A_4 = \tfrac{1}{2}\frac{\partial^2 I_a}{\partial E_a^2} = \frac{1}{2r_{ga}^2}\frac{\partial r_{ga}}{\partial E_a}$$

Substituting in (14) from (16)

$$i_p = \frac{g_a r_{ga}^3}{(R+r_{ga})^3}\, e\left\{\left(\frac{r_{ga}+R}{r_{ga}}\right)^2 - \frac{1}{2r_{ga}^2}\frac{\partial r_{ga}}{\partial E_a} Re + \ldots\right\}$$
$$+\tfrac{1}{2}\frac{\partial_{ga}}{\partial E_a}\frac{r_{ga}^6}{(R+r_{ga})^6}\, e^2\left\{\left(\frac{r_{ga}+R}{r_{ga}}\right)^2 - \frac{1}{2r_{ga}^2}\frac{\partial r_{ga}}{\partial E_a} Re + \ldots\right\}^2 + \ldots + \ldots \tag{17}$$

If the range over which the mutual conductance g_a is constant is chosen as the working range of the tube, then Equation (17) reduces to

$$i_p = \frac{g_a r_{ga}^3}{(R+r_{ga})^3}\, e\left\{\left(\frac{r_{ga}+R}{r_{ga}}\right)^2 - \frac{1}{2r_{ga}^2}\frac{\partial r_{ga}}{\partial E_a} Re + \ldots\ldots +\right\}. \tag{18}$$

By definition $r_{ga} = \dfrac{\partial E_a}{\partial I_a}$ *i.e.*, the reciprocal of the slope of the a grid current—a grid bias curve. If the working range of the tube is further restricted to include only that range of biases for which this slope is constant, then

$$\frac{\partial r_{ga}}{\partial E_a} = 0,$$

and Equation (18) reduces to

$$i_p = \frac{g_a r_{ga}}{R+r_{ga}}\, e, \tag{19}$$

or, substituting the value of e from Equation (7),

$$i_p = \frac{g_a r_{ga}}{R+r_{ga}}\,\frac{E_{ao}+E_{po}}{R+C}\, R. \tag{20}$$

If C is kept very much larger than R, Equation (20) may be written in the approximate form

$$i_p \doteq \frac{I}{C}\,\frac{g_a r_{ga}}{R+r_{ga}}\,(E_{ao}+E_{po})R. \tag{21}$$

In this equation i_p varies inversely as the resistance of the photo-electric cell, all the other terms having been made constants. Grouping the various constants

$$i_p = \frac{K'}{C} \tag{22}$$

If the photo-electric cell has such characteristics that its resistance varies inversely as the energy falling on it, then

$$L = \frac{K''}{C} \tag{23}$$

where L is the light intensity.
Substituting (23) in (22)

$$i_p = KL \tag{24}$$

That is to say that if the assumptions leading up to this equation can be fulfilled then the circuit is capable of linear amplification.

Eight assumptions were made in obtaining the result given by (24). These were— (i) The circuit theory given at the beginning of this section; (ii) The functions given by Equation (2); (iii) $e_p = e_b = 0$; (iv) Terms of the various series higher than the second are negligible; (v) The mutual conductance of the tube over the working range is constant; (vi) r_{ga} is constant over the working range; (vii) C, the resistance of the cell, is very much larger than R the resistance of the grid circuit; (viii) The resistance of the photo-electric cell varies inversely as the light intensity.

Assumptions (i) and (ii) are based upon generally accepted vacuum tube theory.

Assumption (iii) is realized in practice. The screen grid battery is connected directly across the tube elements. If a sufficiently sensitive output meter is used in the plate circuit the shunt across it need not exceed five ohms. The drop in potential across a resistance of this order, with currents of the magnitude passed by a UY-224 tube, is inappreciable.

Assumption (iv) can be shown to be justified by the rapid convergence of the various series involved, but it is easier to prove the soundness of the assumption through the medium of assumption (v). Equation (17) is the expression into which the various series are combined. The two terms shown are not approximations; all possible terms are included in them. Further expansion of the various series shows that every term of Equation (17) higher than the first contains as a multiplier a derivative of the mutual conductance of some order. If the mutual conductance is to remain constant over the working range then each of these terms vanishes and we are justified in dropping terms, of all series, higher than the second.

Assumption (v) is justified by choosing for the working range of the tube that interval over which the mutual conductance is constant.

Assumption (vi) is that r_{ga} is constant over the working range. This assumption may be shown to be true by experimental measurement.

The resistance of the photo-electric cell was measured under the condition of greatest possible illumination and found to be in excess of 100 megohms.

The value of R used in measuring light of similar intensity was one megohm. In this case the error due to this cause is less than the error in meter reading. The assumption imposes a limit on the amplification which can be obtained in practice.

The cell used in this circuit was of the vacuum type and gave resistance variations proportional to the variations in light intensities.

Equation (24) indicates that only the variation in plate current is important. A bucking battery was added to the circuit to balance out the normal plate current through the output meter and thus allow greater accuracy in reading. The zero light intensity was chosen as that when the photo-electric cell shutter was closed. The bucking battery was adjusted to reduce the output meter reading to zero for this value of light intensity.

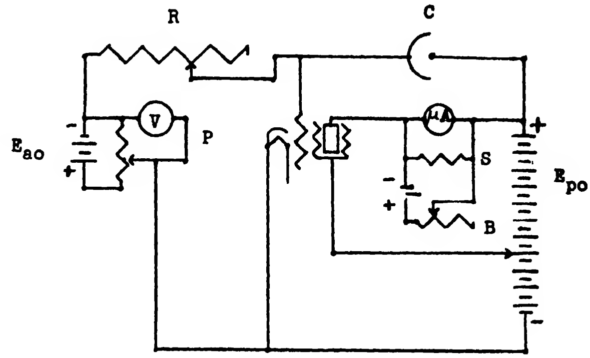

FIG. 2. *Circuit revised to allow more flexible measurements. μA, C, E_{ao}, E_{po}, P, R, S, tube,—as in Fig. 1; B, bucking battery and series resistance; V, voltmeter.*

Fig. 2 is a diagram of the circuit modified to conform with the limitations of the previous paragraphs.

It can be shown that if the negative grid bias of a UY-224 tube exceeds a critical bias of about 1.5 volts, the value of r_{ga} is approximately equal to 15,000 megohms. Since this is very large compared to the highest value of R used, Equation (21) may be still further reduced to

$$i_p = \frac{1}{C} g_a R(E_{ao}+E_{po})$$

or

$$C = \frac{1}{i_p} g_a R(E_{ao}+E_{po}). \tag{25}$$

For each value of R a graph in the form of an equilateral hyperbola may be drawn between C and i_p. The resistance of the photo-electric cell may be taken as an arbitrary unit of light intensity and hence light intensities may be compared regardless of the value of R in the circuit.

The theoretical upper limit of amplification cannot be obtained in practice because of the various limitations imposed in the previous paragraphs. Any of the factors grouped under K' in Equation (22) may be increased to obtain higher amplification. Of these factors R may be most easily controlled. The theoretical upper limit of R can be determined as follows—

$$i_p = \frac{g_a r_{ga}}{R+r_{ga}} \cdot \frac{E_{ao}+E_{po}}{R+C} R, \tag{20}$$

$$\frac{\partial i_p}{\partial R} = \frac{g_a r_{ga}(E_{ao}+E_{po})\,(C r_{ga}-R^2)}{(R+r_{ga})^2\,(R+C)^2}\ .$$

The change in plate current with varying values of R for any given value of C is maximum when

$$\frac{\partial i_p}{\partial R} = 0,$$

i.e., when $R=\sqrt{Cr_{ga}}$.

For most tubes and photo-electric cells the product Cr_{ga} is so large that the maximum value of R will not be reached in practice. The only limit that need be imposed on R therefore is that made necessary by assumption (vii).

The chief advantage of the screen-grid tube in this circuit lies in its stability. Other tubes of higher mutual conductance might be chosen, but all of these, with the possible exception of the space charge pentode, have characteristics which render them inferior to the screen grid tube in the matter of stability.

It can be shown that the UY-224 tube used in a screen grid circuit has an exceptionally high plate impedance. For this reason the plate current is practically independent of the plate potential, provided that the plate potential does not fall from its rated value of 180 volts to within more than 15 volts of the screen potential. It is evident that no attempt need be made to stabilize the plate battery.

Measurement shows that the plate current-screen grid potential curves are all parallel to one another over a wide range of grid bias. Since, in practice, variations in plate current only are of interest, if the mutual conductance of the tube remains constant, the total values of the plate current from which the variation is calculated are not of importance. The parallelism of the various curves indicates that changes in screen potential cause a change in the total value of the plate current without changing the mutual conductance over the range defined by the linear portion of the curve. Stabilization of the screen battery is unnecessary for this circuit.

If the circuit is used in measuring light intensities where the highest possible amplification is necessary, the filament current source should be a high capacity storage battery stabilized by the method suggested by Dearle and Matheson (3). For ordinary measurements where the greatest sensitivity is not necessary the tube may be heated by means of alternating current. The sensitivity of the circuit is increased to a value slightly higher than would ordinarily be necessary, and the sensitivity of the output meter decreased by decreasing the shunt across it.

Since the error due to variations in filament current is independent of the sensitivity of the circuit, the percentage error due to this cause is decreased by this method. In all cases better operation has been obtained by allowing the tube to heat for 30 or 40 min. before attempting to take readings.

While fair results have been obtained with the circuit in an unshielded case, shielding has been found to give much greater stability. Shielding is absolutely necessary if the circuit is to be used in measuring light intensities such that the ultimate sensitivity is required. It has been found that better results are obtained if the shield and the positive end of the B batteries are grounded.

References

1. BRAINERD, J. G. Proc. Inst. Radio Engs. 17: 1006-1020. 1929.
2. CARSON, J. R. Proc. Inst. Radio Engs. 7: 187-200. 1919.
3. DEARLE, R. C. and MATHESON, L. A. Rev. Sci. Instr. 1: 215-226. 1930.
4. HULL, A. W. Phys. Rev. 27: 439-454. 1926.
5. LLEWELLYN, F. B. Bell System Technical Journal, 5: 433-462. 1926.

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THE COEFFICIENT OF VISCOSITY OF SULPHUR DIOXIDE OVER A LOW TEMPERATURE RANGE[1]

By W. W. Stewart[2] and O. Maass[3]

Abstract

The coefficient of viscosity of sulphur dioxide has been measured over a temperature range from 30° to −75°C. The method and apparatus used are similar to those described by Sutherland and Maass (5). All values for the viscosity are referred to air at 23°C. The measured values for the viscosity between 0°C. and 30°C. agree within the experimental error, about 0.1%, with the values published by Trautz and his coworkers. At temperatures below 0°C. the values obtained for the viscosity are important, as they supply data on the viscosity of sulphur dioxide which heretofore have not been available in the literature.

Introduction

This paper discusses the results of the preliminary work on the measurement of the coefficient of viscosity of sulphur dioxide. From a survey of the literature it is quite evident that values for the viscosity of this gas are not available at temperatures below 0°C. These data are essential in order that the validity of equations of state, which depend on viscosity measurements, *e.g.*, the equation of state of Maass and Mennie (2), may be tested over an extended temperature range.

This research is being continued and, when the present apparatus has been reconditioned, may result in slight changes being made in the present values for the viscosity at the lower temperatures, but at present these results are accurate enough to test the validity of the equations of state with the present P.V.T. data available for sulphur dioxide.

In conjunction with this research the density of sulphur dioxide is being determined with a high degree of accuracy at low pressures, in order to obtain the P.V.T. data necessary to study the relationship between the coefficient of viscosity and the gas laws.

The viscosity of sulphur dioxide has been measured by an oscillation disk method. The apparatus and method are similar to those described by

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Sutherland and Maass (5) in a recent publication. The reader is referred to that paper for the complete details of this method.

The only change that has been made in the original apparatus has been in connection with the auxiliary lighting arrangement. The system has been improved by focusing the image of a slit, cut in a brass plate, on the scale. The slit is illuminated by a 200-watt tungsten lamp mounted behind the brass plate in a horizontal position, so that the filament of the lamp is vertical. The light from the slit is concentrated on the mirror by a lens, the image of the illuminated slit being reflected from the mirror and focused on the scale. This image on the scale was about 1 cm. wide with sharply defined edges; it illuminated the markings on the scale sufficiently so that the position of the edge could be observed with an accuracy better than $\pm$ 0.2 mm. when the disk was oscillating.

The sulphur dioxide was purified by condensing it from a tank of c.p. material in a bulb immersed in a carbon dioxide snow-ether bath, and distilling under vacuum in an air-free apparatus until the vapor pressures of two successive fractions were the same. This was indicated on a differential manometer placed between two condensation bulbs, so arranged that they could both be immersed in the same constant-temperature bath. The bath was well stirred and held at -10°C., at which temperature the absolute vapor pressure of sulphur dioxide is about one atmosphere. After three or four distillations the vapor pressures of two successive fractions did not differ by an amount that could be read on the differential manometer. All the gas was tested in this manner before it was distilled into the storage bulbs.

The apparatus was filled with the pure sulphur dioxide at room temperature after flushing out the apparatus several times with the pure gas. Before any observations were made time was allowed for the gas to come to the temperature of the bath. At temperatures below the normal boiling point of the gas, about -10°C., the pressure was reduced. The coefficient of viscosity of a gas is independent of the pressure, so the results obtained at low pressures are comparable with those measured at atmospheric pressure.

Results

The value for the viscosity of the gas is calculated from the expression

$$\eta = \frac{\lambda - x}{Ct},$$

where η is the coefficient of viscosity; λ is the "log. dec." calculated from the observed amplitudes of the oscillating disk; x is the "log. dec." due to the friction of the wire and is a constant; C is a constant depending on the dimensions of the apparatus; and t is the time in seconds for one complete oscillation of the disk.

The value for the constant x has been taken as 0.000173 as determined experimentally by Sutherland and Maass (5).

The constant C is obtained by calibrating the apparatus with a gas, such as air, having a known viscosity. The viscosity of air at room temperature has been determined by many investigators, but the value of the viscosity of air

used in this case was Millikan's (3) most probable value for air at 23°C., namely, 1824.0×10^{-7} C.G.S. units, with an error not greater than 0.1%. To obtain the value of the viscosity of air at any temperature between 17° and 30°C. the expression given by Millikan has been used,

$$\eta_t = 1824.0 \times 10^{-7} - 4.93 \times 10^{-7} (23° - t).$$

The air was purified by passing it through solutions of potassium hydroxide, sulphuric acid and over phosphorus pentoxide.

The average value of C obtained from five completely independent observations was 4.6142. The deviation from the mean value of each determination was less than ± 0.0013 or less than 0.03%.

The results of the measurements of the coefficient of viscosity of sulphur dioxide, using the values of the two constants which have been given above, are shown in Table I. The value λ is obtained from a set of observations each involving at least ten complete oscillations, and in some cases two or more sets of readings have been averaged.

TABLE I

RESULTS OF MEASUREMENTS OF THE COEFFICIENT OF VISCOSITY OF SULPHUR DIOXIDE

Temp., °C.	t, sec.	Press., mm.	λ	$\lambda - x$	$\eta \times 10^{-7}$
29.8	51.100	760	0.031335	0.030604	1298.0
29.5	51.100	760	0.031285	0.030554	1295.8
29.3	51.100	760	0.031282	0.030551	1295.6
20.5	51.095	760	0.030320	0.029589	1255.0
20.4	51.110	760	0.030285	0.029554	1253.2
20.2	51.100	760	0.030252	0.029521	1252.0
0.0	51.055	760	0.028013	0.027282	1158.0
− 5.1	51.065	733	0.027435	0.026704	1133.3
− 6.2	51.070	733	0.027408	0.026677	1132.0
− 7.5	51.055	733	0.027257	0.026526	1126.0
−17.9	51.045	474	0.026261	0.025530	1083.9
−19.2	51.040	450	0.026176	0.025445	1080.6
−20.3	51.030	430	0.026086	0.025355	1076.8
−35.6	51.010	105	0.024592	0.023841	1013.6
−36.5	51.010	105	0.024531	0.023800	1011.2
−75.0	51.000	8	0.020926	0.020195	858.1

TABLE II

COMPARISON OF THE EXPERIMENTAL VALUES OBTAINED IN THIS WORK FOR THE VISCOSITY OF SULPHUR DIOXIDE WITH THOSE OF OTHER INVESTIGATORS

Temp., °C.	$\eta \times 10^{-7}$		
	Observed in present work	Published previously	
0.0	1158.0	1225	Graham, 1846 (1).
		1183	Vogel, 1914 (8).
		1168	Smith, 1922 (4).
14.0	1221.5	1221	Trautz and Weizel, 1925 (6).
17.4	1238.2	1242	Trautz and Zink, 1930 (7).
17.7	1239.0	1239	Trautz and Weizel, 1925 (6).
18.0	1241.0	1253	Smith, 1922 (4).
20.0	1251.0	1380	Graham, 1846 (1).

The average error in the value of the viscosity due to the experimental error is taken to be about 0.1% at any one temperature.

The temperature-viscosity relationship of sulphur dioxide is shown in Fig. 1. The curve shows the viscosity as a function of the temperature and has been plotted by using the values for the viscosity given in Table I. From this curve values for the viscosity have been determined at temperatures at which no experimental value was observed, so that a comparison could be made with the published data on the viscosity of sulphur dioxide.

Table II shows the experimental values for the viscosity of sulphur dioxide in comparison with the published data.

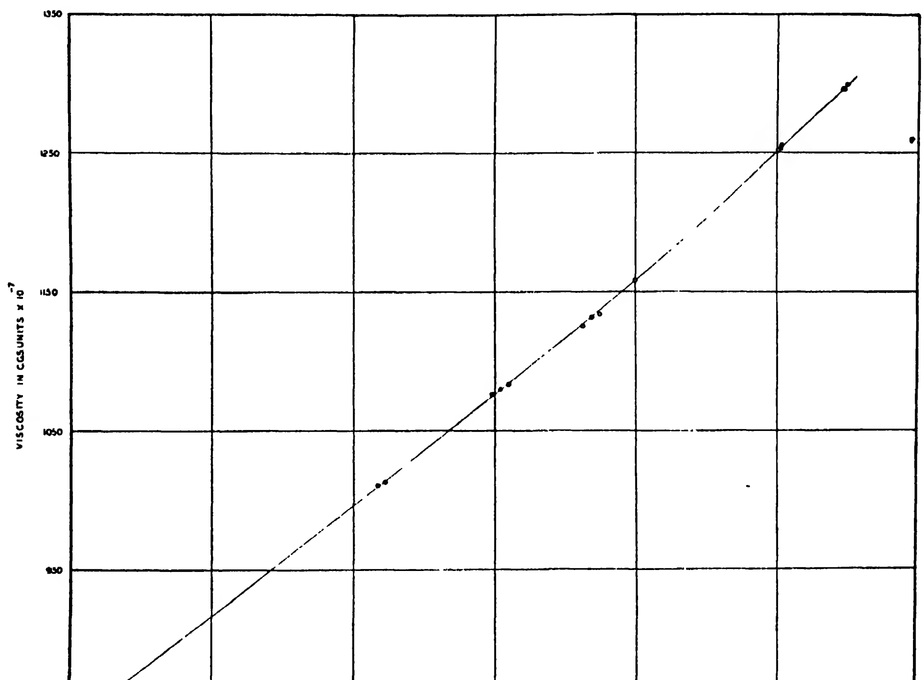

FIG. 1. *Temperature-viscosity relationship of sulphur dioxide.*

At room temperature the observed values for the viscosity of sulphur dioxide are in excellent agreement with the most recent results, those of Trautz and his coworkers. Smith's values at 0°C. and 18°C. are about 1% too large, but the error in Smith's values was as large as 3%. Vogel's value at 0°C. is also too large by about 2.5%. The values published by Graham, which are the values given in most of the tables of physical constants, are undoubtedly too large. At the lower temperatures there are no published data for comparison. The observed values are therefore valuable, as they supply data on the viscosity of sulphur dioxide, which, until the completion of this work, had not been available in the literature.

References

1. GRAHAM, T. Phil. Trans. Roy. Soc. 3: 573. 1846.
2. MAASS, O. and MENNIE, J. H. Proc. Roy. Soc. Lond. A. 110: 198-232. 1926.
3. MILLIKAN, R. A. Ann. Physik, 41: 759-766. 1913.
4. SMITH, C. J. Phil. Mag. 44: 508-511. 1922.
5. SUTHERLAND, B. P. and MAASS, O. 6: 428-443. 1932.
6. TRAUTZ, M. and WEIZEL. W. Ann. Physik, 78: 305-369. 1925.
7. TRAUTZ, M. and ZINK, R. Ann. Physik, (5) 7: 427-452. 1930.
8. VOGEL, H. Ann. Physik, 43: 1235-1272. 1914.

THE CRITICAL TEMPERATURES AND PRESSURES OF THE THREE TWO-COMPONENT SYSTEMS COMPRISED OF CARBON DIOXIDE, METHYL ETHER AND PROPYLENE[1]

By C. A. Winkler[2] and O. Maass[3]

Abstract

The so-called critical temperatures and critical pressures for two-component systems are defined. For the first time three two-component systems have been investigated, involving three components taken two at a time. The three components were propylene, methyl ether and carbon dioxide. An experimental technique involving several new features is described. Accuracy in the determination of both critical temperatures and pressures is claimed. The system sulphur dioxide-methyl ether was also examined and the conclusions of previous investigators regarding compound formation confirmed. The results of the other three systems are analyzed and a theoretical discussion of these results reserved for a later publication.

The Investigation of Critical Phenomena in Gaseous Binary Mixtures

Many and varied have been the investigations on the relationships existing between pressure, temperature and volume, in two-component systems. Among the best publications on the subject are those of Kuenen (7, 8, 9, 10) and Caubet (2, 3, 4). Both of these writers deal with the theoretical aspects of the subject in a well-organized, comprehensive manner. They have no record, however, of having studied the systems carbon dioxide-methyl ether, carbon dioxide-propylene, or methyl ether-propylene. A thorough search of the literature revealed no reference to the last two systems, while the carbon dioxide-methyl ether system has been investigated only to a slight extent.

Briner and Cardoso (1) determined, in part, the pressures for initial and completed liquefaction for three mixtures of carbon dioxide and methyl ether at three different temperatures, and they remarked that the gases do not form a molecular compound. No reference is made, however, to the retrograde condensation which is to be noticed in a binary gaseous mixture at the critical region.

In the investigation of the velocity of reaction by Sutherland and Maass a definite discontinuity was established at the critical temperature. P. V. T. data for two-component systems at the critical temperature are not available in the literature. This investigation is the first step towards P. V. T. determinations in the critical temperature region.

Experimental

Description of Apparatus

The apparatus employed for this investigation was essentially that described by Sutherland and Maass (14); and is diagrammatically represented in

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Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada, with financial assistance from the National Research Council of Canada.
[2] *Postgraduate student, McGill University, and holder of a studentship under the National Research Council of Canada.*
[3] *Professor of Physical Chemistry, McGill University.*

Fig. 1. By careful reconstruction the mechanical difficulties were considerably reduced.

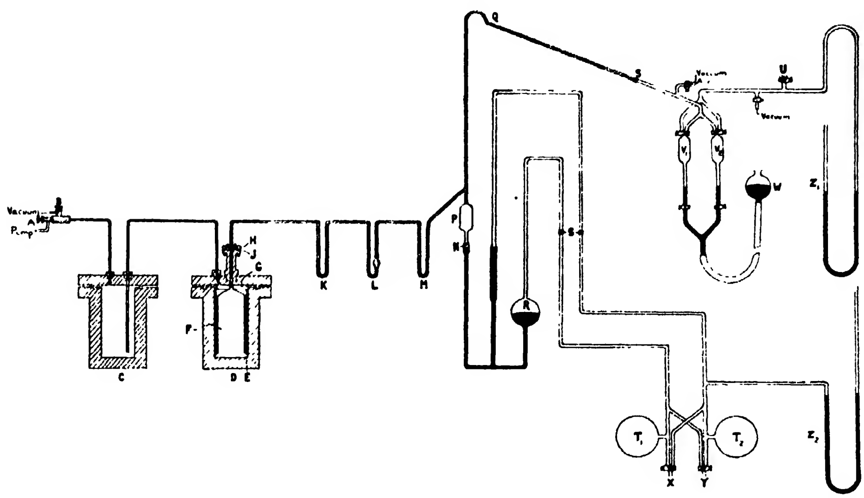

FIG. 1. *Diagram of apparatus.*

The bombs *C* and *D*, machined from shafting steel, and each of a capacity of approximately half a litre, were provided with covers having two apertures as indicated in the diagram. Six half-inch steel bolts served to fasten the cover securely to the bomb. The grooved lead gaskets, shown in the figure, when coated with a thin layer of commercial graphite composition, served to prevent leaks between the bomb and cover, over the entire range of pressures used.

Into the bomb, *D*, a Pyrex glass bell, *F*, was inserted, to which was sealed the heavy-walled Pyrex capillary tubing *KLM* (outside diameter, 7.5 mm.). The capillary tubing passed through a steel pipe screwed into the cover of the bomb, this pipe being 3 in. long, with a bore of 8 mm., and having a 2½-in. flange at its upper end, as shown. A steel cover into which was fitted a steel plate, *H*, and a rubber disk, *J*, was bolted to the flange. By tightening four bolts passing through the cover, the rubber disk was compressed between the steel plate and the lower flange. In this way, the rubber was extruded slightly around the capillary tubing, and forced tightly against it, effecting a seal between the glass and metal portions of the apparatus. Great care had to be taken to prevent unequal pressure on any one portion of the steel plate, otherwise the glass was easily sheared. The seal obtained was quite impervious to mercury, even at pressures up to 125 atm.

To prevent shearing of the glass bell from the capillary tubing as a result of side-sway, at the point where the latter enters the bomb, a steel pipe, *E*, of internal diameter slightly larger than the external diameter of the bell, was screwed into the cover. At intervals around the bottom of this pipe, small

screws were inserted. These could be adjusted to bear lightly against the bell, so that all horizontal motion of the latter was prevented.

Shearing of the bell from the capillary at the same point was also possible by a slight vertical motion. This was especially noticeable at the higher pressures. To overcome this difficulty a thick rubber disk, G, was inserted between the bell and the cover of the bomb, surrounding the capillary tubing. The tubing was also slightly expanded at the point where it passed through the rubber disk in the glass-metal seal. The careful observance of these precautions eliminated a great deal of the mechanical difficulty hitherto encountered in this apparatus.

The bomb, C, was approximately two-thirds filled with carefully purified mercury, the remainder of the volume being filled with oil from the Cailletet compression pump. The bomb, D, was filled with mercury only. This combination of the bombs prevented oil from reaching the bomb, D, ensuring contact of the gaseous mixture with mercury and glass surfaces only.

The bulb, L, in which the observations were made, was of the shape illustrated merely for convenience of operation, as will be evident from the method of manipulation.

The diagram is practically self-explanatory in so far as the remainder of the apparatus is concerned. The Pyrex and soft glass portions of the apparatus were connected by DeKhotinsky joints, S. The volumes T_1 and T_2 served to facilitate pressure control.*

Manipulation of the Apparatus

Preparatory to making a determination, mercury was pumped from the bomb D through the capillary tubing, until it slowly dropped into the volume P. The mercury in M was then frozen in a solid carbon dioxide-acetone mixture, and the remainder of the apparatus evacuated. The gases were admitted individually through the stopcock, U, one gas being permitted to enter the volume V_1 and the other to enter the volume V_2, V_1 and V_2 being of the same capacity. The relative amounts of the gases taken were determined on the open manometer Z_1.

The two-way stopcocks on V_1 and V_2 were then reversed, and the reservoir, W, raised until the mercury reached the point Q. This forced the gases over into the volume, P, after which the stopcocks on V_1 and V_2 were closed. In this manner the gases were contained between the two mercury columns in the capillary tubing, and the mercury in P. Thus, contamination of the mercury in the bombs by traces of stopcock lubricant was avoided.

The mercury in the seal, M, was then permitted to liquefy, and the pressure on the valve, B, reduced simultaneously with the application of a slight pressure to the reservoir R through the stopcock at X. The mercury, rising in P, forced the gas into the glass bell in the bomb D. The mercury from P was permitted to pass along the capillary tubing until it dropped through the smaller into the larger expansion of the bulb at L, whereupon the mercury at

* *The apparatus, as diagrammatically represented, is also intended for future investigations of somewhat different nature, and includes some features which could be dispensed with for the present purpose.*

M was again rapidly frozen. The small amount of mercury remaining in the larger bulb could be drawn into the bomb by first compressing slightly the gases in the bulb, followed by a sudden expansion. The gaseous mixture could then be compressed to the desired extent by means of the Cailletet pump. The pressures were determined on a calibrated Bourdon gauge.

The temperature of the compressed gaseous mixture was controlled by an electrically heated, well-agitated bath of glycoline oil. A lamp-bank resistance in parallel with the heating element gave any desired rate of temperature increment.

Preparation and Purification of the Gases

The carbon dioxide was obtained by sublimation of the solid. The sublimate was passed over phosphorus pentoxide and condensed in a bulb immersed in a solid carbon dioxide-acetone freezing mixture maintained under reduced pressure. This method was adopted to produce slow condensation of the gas, thus avoiding, to a large extent, the occlusion of an appreciable quantity of air in the condensate. The solid so obtained was resublimed and the gas further dried over phosphorus pentoxide, after which it was admitted to the storage flask, which previously had been carefully flushed with carbon dioxide and evacuated.

The critical temperature of the gas was determined as a check on the purity. A value of 31.2°C. was obtained, indicating a sufficiently high degree of purity for the present investigation.

The methyl ether was prepared by the treatment of methyl alcohol with concentrated sulphuric acid. The acid was heated to 135°C. and the alcohol introduced under the surface, a procedure which was found to minimize the loss of methyl ether. The temperature was maintained at approximately 135°C., and the distillate, after passing through a water-jacketed condenser which removed a considerable quantity of alcohol, was passed through concentrated sulphuric acid saturated with methyl ether, and finally through anhydrous calcium chloride, which served to remove traces of alcohol. The methyl ether was finally condensed, using a solid carbon dioxide-acetone mixture. It was then fractionated at a low temperature, the middle fraction being passed over phosphorus pentoxide and condensed in a bulb fitted with a stirrer actuated by a solenoid. The ether was subjected to three further fractionations before being admitted to the carefully flushed and evacuated storage flask.

The critical temperature of the methyl ether thus prepared was found to be 126.2°C., a value in substantial agreement with that of Tapp (15), who, with an apparatus differing from that of the authors, obtained a value of 126.1°C. for various samples prepared by the above method. The critical pressure was found to be 53.0 atm.

Propylene was prepared by the dehydration of isopropyl alcohol over alumina at 360°C. and purified by low temperature fractionation, as described by Coffin and Maass (5, 6) and Maass and Wright (11, 12). The purity of the propylene was checked by vapor pressure measurements during fractionation,

and by determination of the critical temperature, for which a value of 92.9°C. was obtained. Since this is in agreement with that found by Maass and Wright (11) who report a value of 92.1°C., the gas was used without further purification.

Determination of the Vapor Pressures of the Binary Mixtures

When the gaseous mixture had been brought into the glass bell contained in the bomb *D*, and the mercury seal, *M*, frozen, pressure was applied until partial liquefaction occurred. The temperature of the oil bath surrounding the bulb at *L* was then slowly increased, care being exercised to prevent appreciable lag of the temperature inside the bulb relative to that of the bath. As the temperature was raised, the pressure being maintained at a constant value, the amount of liquid slowly decreased. The temperature at which the liquid phase just disappeared was regarded as that corresponding to the pressure of the vapor as registered on the Bourdon gauge. The observations were considerably facilitated with the aid of a telescope, which enabled minute traces of liquid to be seen.

The Bourdon gauge was calibrated, over a range up to 70 atm., against the vapor pressure of pure carbon dioxide, and the necessary corrections, obtained from a calibration curve, applied to all subsequent pressure readings.

After each successive observation, the pressure was increased slightly until liquid again appeared, and the corresponding temperature for disappearance of the meniscus determined as before.

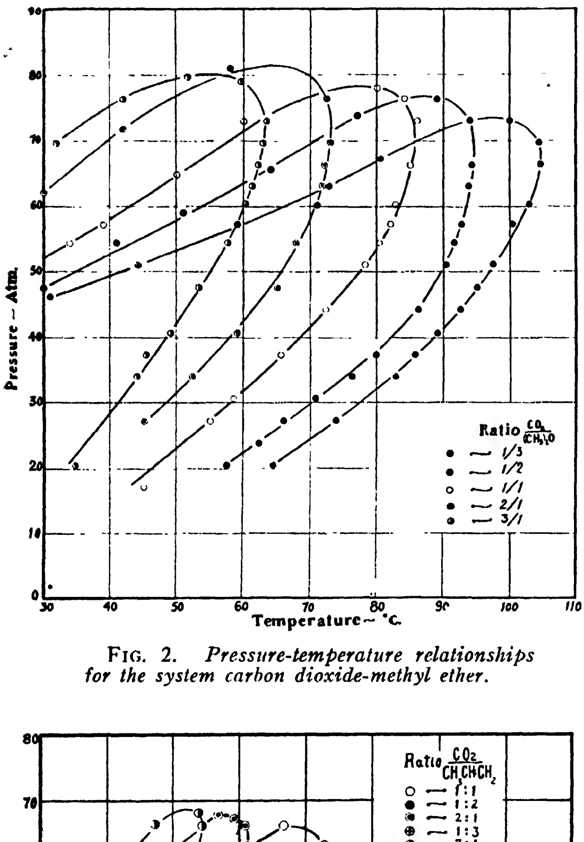

FIG. 2. *Pressure-temperature relationships for the system carbon dioxide-methyl ether.*

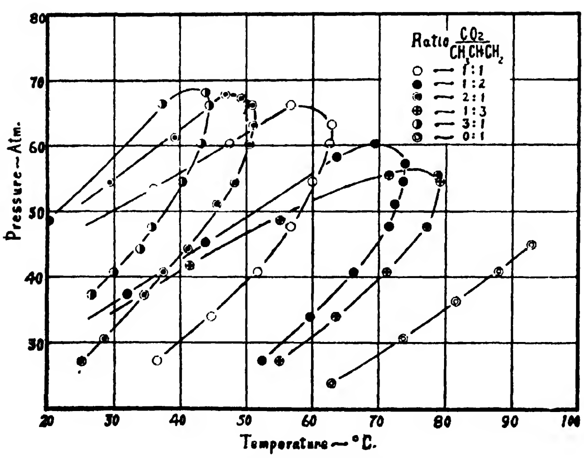

FIG. 3. *Pressure-temperature relationships for the system carbon dioxide-propylene.*

The pressures corresponding to complete liquefaction or condensation at various temperatures were also determined for each mixture investigated. This was done by maintaining the temperature at a given value, and slowly increasing the pressure until only a trace of gaseous mixture remained. It was absolutely essential that the system be permitted to attain equilibrium. To ensure this, the cross hair in the eyepiece of the telescope was focused on the boundary surface of the gas bubble. If, after some time, no increase or decrease in the size of the bubble had occurred, it was assumed that equilibrium had been attained.

In all cases, the temperatures were ascertained on standardized thermometers with an error not exceeding 0.1°C.

The results obtained for a mixture of a given ratio of components were checked, using a second mixture of the same composition. Duplicate determinations were required to agree within 1.0°C. and 2 atm. in the case of the carbon dioxide and methyl ether curves, these being the first to be determined. For the mixtures of carbon dioxide and propylene, and those of propylene and methyl ether, it was found that agreement within 0.5 and 1 atm. could be obtained, by measuring out larger amounts of the gases, although maintaining the desired ratio.

Results and Discussion

The results obtained for the determinations of the vapor pressures, and the pressures required for complete condensation, at various temperatures, are given in Tables I-VI, and graphically represented in Figs. 2-4, inclusive.

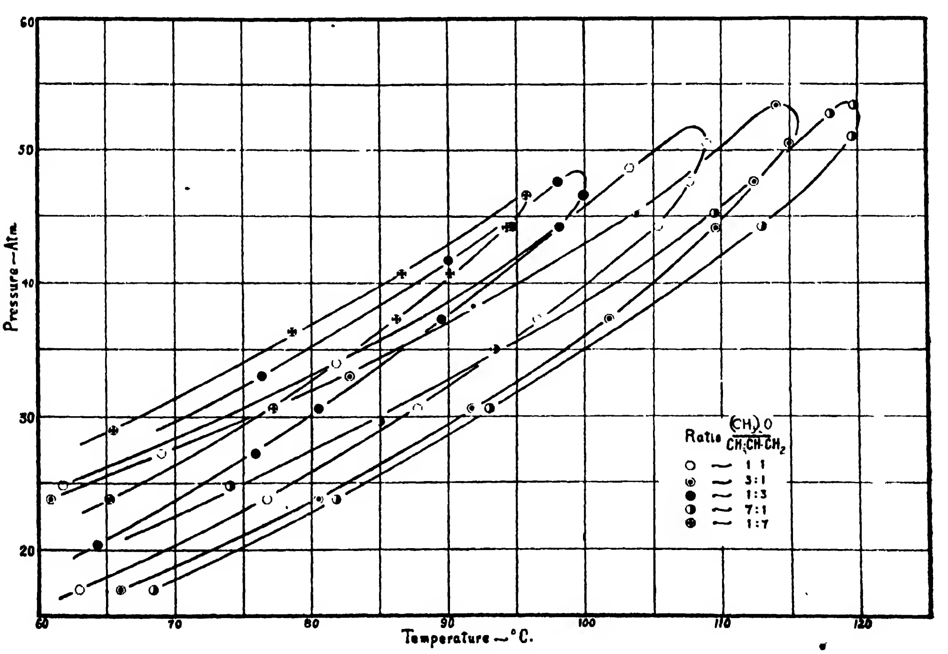

FIG. 4. *Pressure-temperature relationships for the system methyl ether-propylene.*

TABLE I

THE VAPOR PRESSURE-TEMPERATURE RELATIONSHIPS FOR THE SYSTEM CARBON DIOXIDE-METHYL ETHER

Pressure, atm.	Temperature (°C.) for disappearance of meniscus for ratio of carbon dioxide to methyl ether of:				
	1:1	1:2	2:1*	1:3	3:1
17.0	45.1	....	....	...	....
20.4	....	57.5	....	64.5	34.9
23.8	....	62.3	...	....	....
27.2	55.0	66.0	45.2	73.8	....
30.6	58.5	70.8	...	....	....
34.0	....	76.3	52.3	82.8	44.0
37.3	65.6	79.9	...	85.7	45.4
40.7	....	....	59.0	89.1	49.1
44.2	72.3	86.2	....	92.5	....
47.6	....	..	65.0	95.0	53.3
51.0	78.2	90.4	..	97.5	....
54.4	80.3	91.6	67.8	...	57.6
57.2	82.0	92.6	..	100.4	....
60.2	82.7	...	71 0	103.0	60.3
63.1	....	93.8	71.8	....	61.2
66.2	85.0	94.3	72.1	104.7	62.2
69.6	. .	. . .	73.0	104.5	62.9
73.0	86.0	94.0	.	100.0	63.4
76.4	84.1	89.0	72.4?	....	54.0?
78.0	70.0	....	..	....	....

* *This curve was not checked.*

The upper portion of each curve represents the trend of the pressure values for complete condensation at the temperatures indicated, while the lower portion represents the increase in vapor pressure of the mixture with increasing temperature. It is to be noticed that the condensation curve and vapor pressure curve are really continuous.

If, for a given pair of components, a line is drawn tangentially to all the curves at their upper extremities, the point of contact of this line (the critical line) with a given curve is known as a plait-point on that curve. The corresponding temperature may be termed the plait-point temperature.

TABLE II

THE CONDENSATION PRESSURE-TEMPERATURE RELATIONSHIPS FOR THE SYSTEM CARBON DIOXIDE-METHYL ETHER

Ratio of carbon dioxide to methyl ether									
1:1		1:2		2:1		1:3		3:1	
Temp. °C.	Press. atm.	Temp. °C.	Press. atm.	Temp. °C.	Press. atm.	Temp. °C.	Press. atm.	Temp. °C.	Press. atm.
34.0	54.4	41.0	54.4	30.0	62.2	31.0	46.2	32.0	69.6
39.0	57.2	51.0	59.0	42.2	71.8	44.0	51.0	42.0	76.4
50.0	64.8	64.0	65.5	58.0	81.0	59.0	57.2	51.6	79.7
60.0	73.0	77.0	73.8	....	....	72.8	63.1	59.6	79.0
...	...	....	....	....	....	80.5	67.2	....	....

The temperature corresponding to a tangent drawn parallel to the pressure axis at the point of maximum abscissa of a given curve has been termed the critical contact temperature. Above this temperature liquefaction was found to be impossible, regardless of the magnitude of the pressure applied. As Kuenen (7, 8, 9, 10) and Caubet (2, 3, 4) have pointed out, however, this point does not represent a true critical temperature, since such a mixture possesses a critical region. The considerations leading up to this conclusion may be briefly outlined, as applied to the observations made in the present work.

At temperatures below the plait-point temperature, condensation was regular, inasmuch as the quantity of liquid phase increased regularly during compression, until the mixture was completely liquefied. Between the plait-point and critical contact temperatures, the condensation was characterized initially by an increase in the amount of liquid present as the pressure was

TABLE III

THE VAPOR PRESSURE-TEMPERATURE RELATIONSHIPS FOR THE SYSTEM CARBON DIOXIDE-PROPYLENE

Pressure, atm.	Temperature (°C.) for disappearance of meniscus for ratio of carbon dioxide to propylene of:				
	1:1	1:2	2:1	1:3	3:1
27.2	36 5	52.5	25.1	55 1	18.3
34.0	44 8	59.7	.	63.6	....
37.3	.		34.6		26.6
40.7	51.8	66 3	37.4	71 2	30.0
44.2	.		41.2		34 0
47.6	56 8	71.5		77.2	35.7
51.0		72 3	45 6		
54.4	60.0	73.6	48.3	79.1	40.3
55.4	....	.	..	78.8	....
57.2	. . .	73.9	.	. .	.
60.2	62 6	....	50.5	....	43.3
63.1	63.0	....	51.1	.. .	...
66.2	. 56.8	....	50 9	..	44.4
67.2	....	....	49.3	.	....
68.2	....	....	....	....	44.0

TABLE IV

THE CONDENSATION PRESSURE-TEMPERATURE RELATIONSHIPS FOR THE SYSTEM CARBON DIOXIDE-PROPYLENE

Ratio of carbon dioxide to propylene									
1:1		1:2		2:1		1:3		3:1	
Temp., °C.	Press., atm.	Temp., °C.	Press., atm.	Temp., °C.	Press., atm.	Temp., °C.	Press., atm.	Temp., °C.	Press., atm.
36.0	53.4	32.0	37.3	29.4	54.4	41.4	40.7	20.2	48.6
47.5	60.2	43.8	45.2	39.2	61.2	55.2	48.6	37.2	66.4
....	....	63.6	58.2	46.9	67.9	71.5	55.4	....	....
....	....	69.4	60.2	....	....	....	....	.	....

TABLE V

THE VAPOR PRESSURE-TEMPERATURE RELATIONSHIPS FOR THE SYSTEM METHYL ETHER-PROPYLENE

Pressure, atm.	Temperature (°C.) for the disappearance of the meniscus for the ratio of methyl ether to propylene of:				
	1:1	1:3	3:1	1:7	7:1
17.0	63.0	. . .	66.0	....	68.4
20.4	....	64.3	. .	. . .	.
23.8	76.7		80.5	65.2	81.8
27.2	....	75.9	.	.	....
30.6	87.7	80.5	91 7	77.2	93.1
37.3	96.5	89.5	101.8	86.2	.
40.7	....			90.1	...
44.2	105.4	98.1	109 6	94.2	113.0
46.6	..	99.9		95 6	..
47.6	107.7	.	112.4	. .	..
51.0	108 9	.	115 0		119.6
53.4	..		114 0	.	119.4

TABLE VI

THE CONDENSATION PRESSURE-TEMPERATURE RELATIONSHIPS FOR THE SYSTEM METHYL ETHER-PROPYLENE

Ratio of methyl ether to propylene									
1:1		1:3		3:1		1:7		7:1	
Temp.	Press.	Temp.	Press.	Temp.	Press.	Temp.	Press.	Temp.	Press.
61.8	24.8	76.4	33.0	60 8	23.8	65.5	28.9	74.0	24 8
69.0	27.2	90.0	41.7	82.8	33.0	78.6	36.3	85.0	29 6
81.8	34.0	94.7	44.2	91.8	38.3	86.6	40.7	93.4	35.0
103.3	48.6	98.0	47.6	103.6	44 2	....	.	109.5	45.2
....	. . .	. .	. . .	....		. .	.	118.0	52 7

increased, until the pressure corresponding to the critical point of contact was attained. For pressures greater than this value the liquid phase decreased in amount, and ultimately disappeared. This phenomenon has been called, by Kuenen, retrograde condensation.

It is evident from these considerations that a single critical point cannot be ascribed to a mixture, but that the plait-point and critical contact temperatures may be regarded as limiting a critical region.

The differences existing between the critical temperature as calculated, and the plait-point and critical contact temperatures for the three systems investigated are shown in Fig. 5. The last two named temperatures were ascertained from the corresponding curves in Figs. 2–4. The calculated critical temperatures were determined from the law of mixtures, formulated by Pawlewski (13) as follows:

$$T_c = \frac{nt_c' + (100-n)\, t_c''}{100},$$

where T_c is the critical temperature of the mixture containing $n\%$ by weight

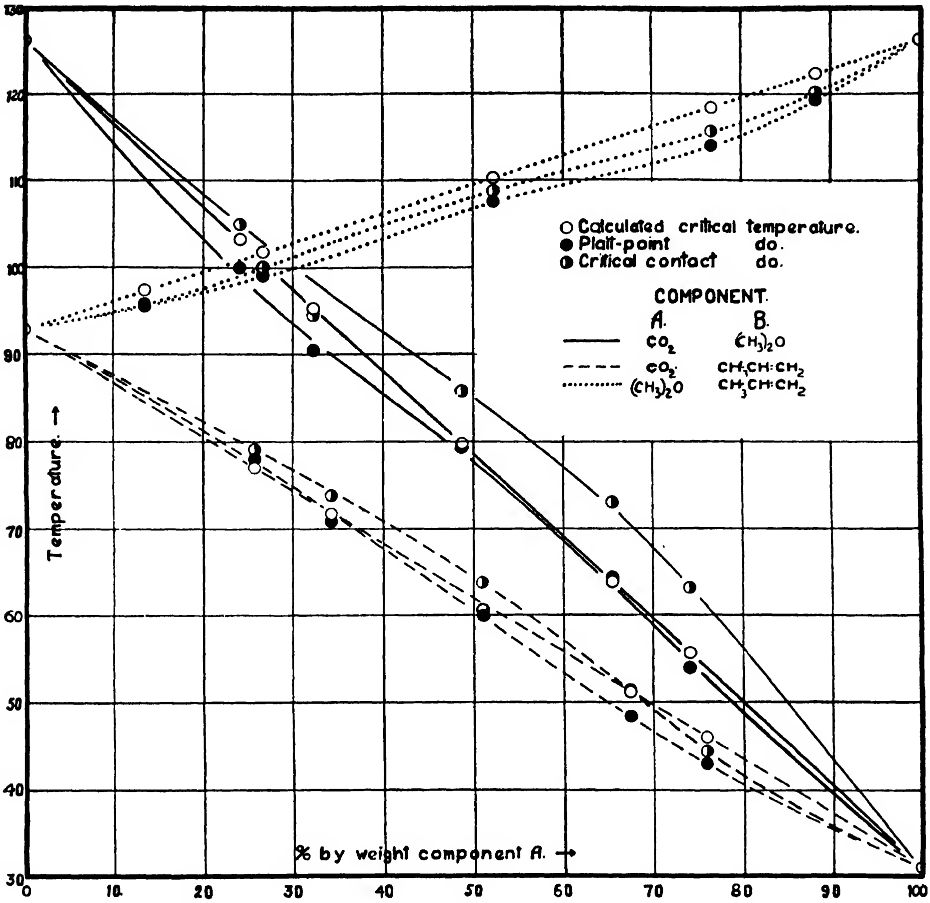

FIG. 5. *Comparison of the calculated critical temperature, and the plait-point and critical-contact temperatures, for the systems carbon dioxide-methyl ether, carbon dioxide-propylene and propylene-methyl ether.*

of one component whose critical temperature is t_c', and (100-n)% by weight of a second component whose critical temperature is t_c''.

The results confirm the contention of Kuenen that considerable deviations from the mixing rule may be encountered. This is rather to be expected, since the law of mixtures does not take into consideration the phenomenon of retrograde condensation.

In Fig. 6 are plotted the plait-point and critical-contact pressures. Here again are to be noted large discrepancies from a straight line relationship.

The fact that retrograde condensation was a prevalent factor in the systems carbon dioxide-methyl ether, carbon dioxide-propylene and methyl ether-propylene, may be taken to indicate the absence of molecular compound formation in any one of these mixtures. Verification of this conclusion was obtained by using a mixture of sulphur dioxide and methyl ether, which

gases Briner and Cardoso (1) have shown to form a molecular compound of the type $(CH_3)_2O.SO_2$.

The sulphur dioxide was obtained by carefully drying the commercial gas, free from sulphur trioxide, and subjecting it to a series of low temperature fractionations as in the case of propylene.

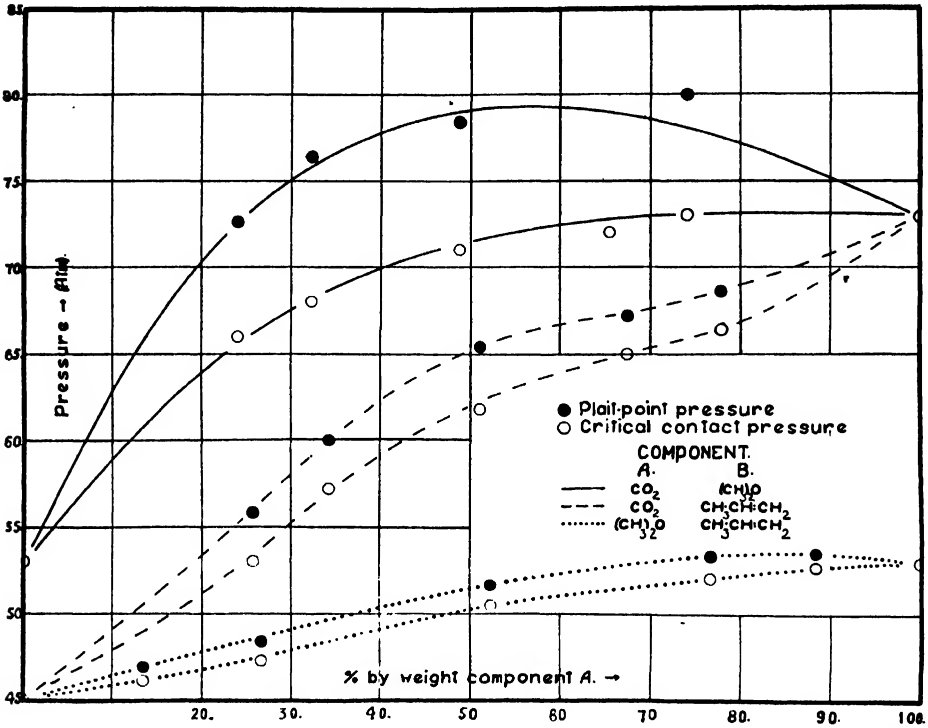

FIG. 6. *The plait-point and critical-contact pressures of the systems carbon dioxide-methyl ether, carbon dioxide-propylene and propylene-methyl ether.*

It was found that for a 1:1 mixture of the gases, the curve of Briner and Cardoso (1) was checked to within 0.5°C. and 1 atm. through the entire range. It proved to be impossible to detect retrograde condensation for this mixture, and the pressure for complete liquefaction was practically the same as that for the initial appearance of a liquid phase. This behavior is characteristic of a pure component.

It is interesting to note that as the difference between the critical temperatures of the components of a mixture diminishes there is a corresponding decrease in the difference between the vapor pressures and the pressures for complete condensation, as may be seen by a comparison of Figs. 2, 3, and 4. Coincident with this decrease, there is to be noticed a smaller critical region.

Fig. 5 also reveals many points of interest. For the system carbon dioxide-methyl ether, the plait-point temperature curve lies consistently below the curve for the calculated critical temperature, but tends to approach the latter

in the region of a 1:1 composition. The maximum deviation is approximately 4°C. On the other hand, the critical contact temperature curve lies consistently above that for the calculated critical temperature, and shows a maximum deviation of approximately 8°C. from the latter in the region of 1:1 composition.

In the case of the methyl ether-propylene system both the plait-point and critical-contact temperature curves lie below the curve of the calculated critical temperature, the former curves paralleling one another closely. Both show tendencies to deviate to the largest extent on either side of a 1:1 composition.

The system carbon dioxide-propylene is striking in so far as both the plait-point and critical-contact temperature curves intersect the calculated critical temperature line, but at opposite sides of a 1:1 composition. It is noticed that the plait-point temperatures are above the calculated values for amounts of propylene in excess of approximately one-third of the total amount of gas. When the mixture becomes richer in carbon dioxide than corresponds to a ratio 1:2, the plait-point curve falls below the calculated line, and remains below until the pure carbon dioxide point is attained. The maximum deviation from the calculated values occurs in the region of approximately 75% carbon dioxide.

The critical-contact temperature curve parallels that for the plait-point temperatures, but passes above the calculated line for all mixtures containing less than approximately 67% carbon dioxide. Above this composition the critical-contact temperature falls below the calculated critical temperature, and remains thus until the pure carbon dioxide point is reached. The maximum deviation above the calculated line occurs at a composition of about 45% carbon dioxide, while the maximum deviation below the calculated values occurs at about 82% carbon dioxide.

Fig. 6 also shows several features of considerable interest.

In the case of carbon dioxide-methyl ether there is a definite maximum in the plait-point pressures, this maximum being 76.6 atm., *i.e.*, above the critical pressure of either of the components. This maximum occurs at a composition of approximately 55% carbon dioxide. The critical-contact pressures do not attain a maximum at compositions intermediate between the pure components, but increase steadily to the critical pressure of pure carbon dioxide. The maximum discrepancy between the two curves is approximately 16 atm. at a composition of about 50% carbon dioxide. Neither curve shows points of inflexion, but throughout their course both are above the straight line joining the critical pressure of the pure components.

The system carbon dioxide-propylene exhibits inflexions in the curves of both the plait-point and critical-contact pressures. Although there is a continual increase in both pressures as the percentage of carbon dioxide is increased, there is a tendency for the values to fall off after a concentration of 50% carbon dioxide until 67% is reached, after which they increase again to the critical pressure of carbon dioxide. The maximum discrepancy is approximately 7 atm.

For the system methyl ether-propylene there seem to be indications of inflexions, though these are not very definite. The increase is quite steady until a composition of approximately 75% methyl ether is attained, when a slight decrease in the plait-point pressure is in evidence, although the critical contact pressure appears to increase more steadily. A slight inflexion also occurs in the region of about 30% of methyl ether.

In a future communication the authors hope to correlate the observed differences with the intrinsic properties of the components of each of the systems.

References

1. Briner, E. and Cardoso, E. J. chim. phys. 6: 641-680. 1908.
2. Caubet, F. Z. physik. Chem. 40: 257-367. 1902.
3. Caubet, F. Z. physik. Chem. 43: 115-117. 1903.
4. Caubet, F. Z. physik. Chem. 49: 101-116. 1904.
5. Coffin, C. C. and Maass, O. Can. J. Research, 3: 526-539. 1930.
6. Coffin, C. C. and Maass, O. Ph. D. thesis, McGill University.
7. Kuenen, J. P. Phil. Mag. (5) 40: 173-194. 1895.
8. Kuenen, J. P. Z. physik. Chem. 11: 38-48. 1893.
9. Kuenen, J. P. Z. physik. Chem. 24: 667-696. 1897.
10. Kuenen, J. P. Z. physik. Chem. 37: 485-489. 1901.
11. Maass, O. and Wright, C. H. J. Am. Chem. Soc. 43: 1098-1111. 1921.
12. Maass, O. and Wright, C. H. J. Am. Chem. Soc. 46: 2664-2673. 1924.
13. Pawlewski, B. Ber. 16: 2633-2636. 1883.
14. Sutherland, B. P. and Maass, O. Can. J. Research, 5: 48-63. 1931.
15. Tapp, J. S. Private communication.

REACTIONS OF ETHYL ALCOHOL ON NICKEL-CHROMIUM CATALYSTS[1]

BY E. H. BOOMER[2] AND H. E. MORRIS[3]

Abstract

A series of catalysts containing nickel and chromium has been prepared and their action on mixtures of ethyl alcohol and water studied. The most active catalysts are prepared by precipitation of the metals as hydroxides or in combination as nickel chromate. The activity of the catalysts depends very much on the nature of the treatment accorded them in preparation. The action of the nickel always predominates but it is more sensitive than chromium to vigorous treatment. The catalysts generally lose some of their activity with use, the nickel more so than the chromium, and the dehydrating efficiency of the catalyst may rise. Secondary reactions, with the production of carbon and complex organic liquids, usually occur, both of which result in carbon dioxide production.

Introduction

The catalytic properties of a given substance or mixture of substances may be examined readily by the use of a suitable reaction. Of a number of reactions available for such studies, one in particular is of wide application, namely, the decomposition of ethyl alcohol. It is well known (13, 17) that most metals and oxides may be classified as to their catalytic properties by this reaction. There are two main types of reaction involving dehydrogenation and dehydration as shown in Equations (1) and (2).

$$C_2H_5OH = CH_3CHO + H_2 \quad (1)$$

$$C_2H_5OH = C_2H_4 + H_2O \quad (2)$$

In general the classification includes those substances promoting dehydrogenation alone, dehydration alone, and those substances with the properties of a mixed catalyst in that both reactions are promoted.

Although the literature regarding the decomposition of ethyl alcohol over simple catalysts is extensive, comparatively little has been done with mixtures of catalysts. Such mixtures are of interest because of their relation to promoted and supported catalysts which have been widely investigated (12). Furthermore the study of reactions under pressure is characterized by the use of complex catalysts, frequently mixtures.

In connection with other work a study of the decomposition of ethyl alcohol and ethyl alcohol-water solutions in the vapor phase over mixtures of catalysts was desirable. This paper contains the results obtained with a large variety of binary mixtures, and a few supported and promoted mixtures, of nickel and chromium. The effects of composition, methods of preparation and reduction procedures have been investigated and discussed.

Literature Review

Before examination of the present results it may be well to consider some of the more evident properties of the two individual catalysts. In general nickel

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Contribution from the chemical laboratories of the University of Alberta, Edmonton, Canada, with financial assistance from the National Research Council of Canada and the Research Council of Alberta.
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is a very active, but sensitive, dehydrogenating catalyst. Chromium, while not as active as nickel, is comparatively resistant to deactivation by harsh treatment. The reaction over chromium may vary from 40 to 90% dehydrating (10) depending upon the method of preparation.

The dehydrogenating action of nickel is well known and its action upon ethyl alcohol has been reported frequently. Sabatier and Senderens (19) observed that reduced nickel acted violently at relatively low temperatures to produce hydrogen and acetaldehyde. Beginning at about 150° C. the reaction was rapid and at 178° C. the gaseous products were 23% carbon monoxide, 29% methane and 48% hydrogen, the two former products being produced by the decomposition of acetaldehyde with subsequent hydrogenation of some of the carbon monoxide to methane. At temperatures about 50° C. higher the decomposition of carbon monoxide commenced and increased rapidly until, at a temperature of 330° C., the gas consisted of 19.5% carbon dioxide, 60.7% methane and 19.8% hydrogen.

Mond, Langer and Quincke (11) studied the variation in activity of different forms of nickel and found that the massive metal was less active than metal reduced from the oxide. The decomposition of carbon monoxide on massive nickel was not promoted until a temperature of 350° C. was reached. Adkins and Lazier (1) noted a variation in the activity of reduced nickel catalysts depending upon the nature of the reducing agent employed. Catalysts reduced with ethyl alcohol at 350°-420° C. were more active than similarly prepared samples reduced with hydrogen at 300°-400° C. These experimental results indicate quite clearly the activity of nickel in the catalytic decomposition of ethyl alcohol and show its marked dehydrogenating action.

The action of chromium has not been investigated to the same degree, but it is known that most non-reducible oxides are mixed catalysts promoting both dehydration and dehydrogenation at the same time. Sabatier and Mailhe (16) mention that Cr_2O_3 is a mixed catalyst promoting reactions with the simultaneous formation of ethylene, water, hydrogen and acetaldehyde. These workers also found that the crystallized oxide produced no gas at 350° C. and only a very small quantity of pure hydrogen at 400° C. (17). Lemoine (10) investigated the action of chromium catalysts on ethyl alcohol in more detail and found that the sesquioxide, prepared by dehydrating the blue precipitated hydroxide, produced a gas containing 91% ethylene and that after calcination at 500° C. this same oxide produced only 40% ethylene. Sabatier (15) concluded that the only form of chromium suitable for the dehydration of ethyl alcohol is the sesquioxide obtained by drying the precipitated hydroxide below 350° C. Calcination of the oxide resulted in a catalyst of equal dehydrating and dehydrogenating power. In the light of these results it follows that the dehydrating action of chromic oxide is pronounced and ought to be evident even in the presence of another powerful catalyst of different action.

The effect of water upon the decomposition of alcohol has also been investigated. Armstrong and Hilditch (3) using a copper catalyst found that the presence of water inhibited the decomposition of the aldehyde produced.

Hoover and Rideal (6) observed that water had a marked effect upon the ratio of dehydrating and dehydrogenating activity at the surface of a thoria catalyst. Lazier and Adkins (9) noted that water increased the carbon dioxide and hydrogen percentages in the gases produced. In a more recent paper Russell and Marschner (14) concluded that the presence of water increases the amount of alcohol decomposed and decreases the secondary decomposition of the aldehyde.

Discussion

In regard to the action of nickel and chromium mixtures, as shown by the present work, it may be said at once that the promoter action is slight or absent altogether. In general the mixtures behave as two chemical individuals, each having its characteristic properties modified by their relative concentrations and by the method of preparation. The effects due to the mixture as such are confined largely to the reactions subsequent to the initial decompositions. The relative activity of the two catalysts is easily determined and is of interest inasmuch as single catalysts may be classified by comparison of the actions of the different pairs of catalysts containing a common constituent. For example, it has been shown (5) in the case of a catalyst containing copper and chromium on silica, that the action of the chromium was greater than that of the copper at low temperatures, but as the temperature increased, the copper became more active relative to the chromium. The work reported here indicates that nickel is more active than chromium, but with vigorous treatment the relative activity of the two catalysts is shifted in favor of the chromium. From these results the apparent order of resistance of catalysts to thermal deactivation is, copper, chromium, nickel. This conclusion is verified to some extent by results which indicate that the depressing effect of water on the decomposition of alcohol is not as marked over nickel and chromium mixtures as Armstrong and Hilditch (3) found to be the case with copper.

The change in properties of catalysts with change in reaction temperature is of common occurrence and is evident in these mixed catalysts, there being a well-defined temperature region in which a marked change in the reactions occurs. This may be due to a change in the activity of the nickel or to a new effect which can be attributed to a mixture of the catalysts as a whole. The dehydrating action of the chromium is always evident and no abrupt change is apparent in this reaction.

Preparation of Catalysts

The present study involved the examination of the action of 20 catalysts. They varied in composition and in method of preparation as described below.

Catalyst No. 38 was prepared by the precipitation of nickel chromate from a nickel nitrate solution by potassium chromate. The nickel chromate was washed free of electrolytes and dried at 110° C.

Catalyst No. 46 was an equimolar mixture of c.p. samples of nickelous oxide and chromic oxide, wetted with water, ground, and dried at 110° C.

Catalyst No. 49 was prepared from the hydroxides of nickel and chromium precipitated together from an equimolar solution of the nitrates with sodium

hydroxide. The washed precipitate was dried, pulverized, triturated with water and dried at 110° C.

Catalysts Nos. 51 to 57 were prepared from nickel and chromium hydroxides precipitated with sodium hydroxide from the nitrates. The washed hydroxides were mixed wet in various proportions, dried at 110° C. and analyzed. The series contained the following percentages of nickelic oxide: 97, 86, 80, 66, 50, 40 and 9.

Catalyst No. 58 was prepared by the method used for Nos. 51-57 but with the substitution of ammonium hydroxide for sodium hydroxide. This catalyst was 53% nickelic oxide.

Catalyst No. 59 was pure chromic oxide prepared by precipitation of chromic hydroxide by potassium hydroxide from a solution of chromic nitrate. The precipitate was washed thoroughly and dried at 110° C.

Catalyst No. 60 was pure nickelic oxide prepared by the method used for catalyst No. 59 with the substitution of nickelic nitrate for chromic nitrate.

Catalyst No. 61 was prepared by soaking long fibre asbestos in an equimolar solution of the nitrates followed by ignition at 1000° C.

Catalyst No. 62 was prepared by evaporating an equimolar solution of nickel and chromium nitrates to dryness and igniting the resulting mass at 1000° C.

Catalysts Nos. 63 and 65 were prepared on porcelain chips by soaking them in an equimolar solution of the nitrates with subsequent boiling in potassium hydroxide solution. Catalyst No. 63 was drained and dried at 110° C. It contained potassium hydroxide as well as nickel and chromium oxides. Catalyst No. 65 was washed thoroughly to remove electrolytes and dried.

Catalyst No. 67 was prepared as in the case of No. 61 with the substitution of porcelain chips for asbestos.

Catalyst No. 68 was prepared by soaking an alumina gel in a solution of nickel and chromium nitrates and dried by ignition.

Catalyst No. 69 was prepared by treating alumina gel, that had been soaked in a solution of nickel and chromium nitrates, with potassium hydroxide solution. This mixture was filtered, washed and dried.

All catalysts, except where noted in the discussion, received the same treatment. They were subjected to the action of a slow stream of hydrogen for five to six hours at 300°-310° C.

Procedure

A standard apparatus as previously described (5) was used in this work. The alcohol solution was forced from a calibrated reservoir into a vaporizer at 105°-115° C. The vapors were led over the catalyst and the products removed rapidly through a capillary tube. Two condensers were used, the first at 10° C. and the second below 0° C. The total gas yield was measured by a wet test-meter and calibrated flow-meters gave instantaneous rate readings. Temperatures were determined by means of a noble-metal thermocouple placed in a glass well in the centre of the catalyst space. The interior and exterior of the catalyst tube differed in temperature by about 5° C. Gases were sampled over glycerol and analyzed in the usual manner with a Bureau of

Mines apparatus using copper oxide for carbon monoxide and hydrogen and slow combustion in oxygen for methane and ethane.

Unless otherwise stated the rate at which the alcohol solution passed into the catalyst chamber was 0.75 cc. per min. In case of 100% dehydrogenation this would correspond to about 225 cc. of hydrogen per min. with equal amounts of carbon monoxide and methane in the event of complete decomposition of the acetaldehyde produced in the primary reaction. The same figure represents the ethylene in case of dehydration. Any action due to water is not considered.

In view of the fact that an investigation of the gaseous products was the primary object of this work, the liquid products were not thoroughly examined. With these catalysts the liquids were complex mixtures of water, aldehydes, acids and esters. A complete and quantitative analysis was not made.

In the tabulated results the column showing the ratio liquid in/out, refers to the total liquid volumes concerned and in some cases the figure obtained is the result of several experiments.

Results

Of the great number of experiments performed, many were in relation to other investigations. Only those results having direct bearing on the subject under discussion will be presented.

Inspection of the data in Table I shows that in general the series of catalysts Nos. 51 to 57 gave the expected results. The nickel was much more active than the chromium, which is the reverse of the effect of copper in copper-chromium catalysts (5).

Catalyst No. 56 containing 97% of nickelic oxide indicated its activity by a sudden increase in temperature from 300° to 340° C. when the vapor reached it. The gas yield varied with different conditions as shown in the table. As the temperature was raised, the activity as measured by gas flow reached a maximum at 300° C. and decreased above that temperature. A second run was started at 260° C. and in general the gas was richer in carbon dioxide, hydrogen and carbon monoxide and had a lower methane content. The rate of flow was larger and the temperature of maximum activity rose to 350° C. There is an abrupt change in the gas

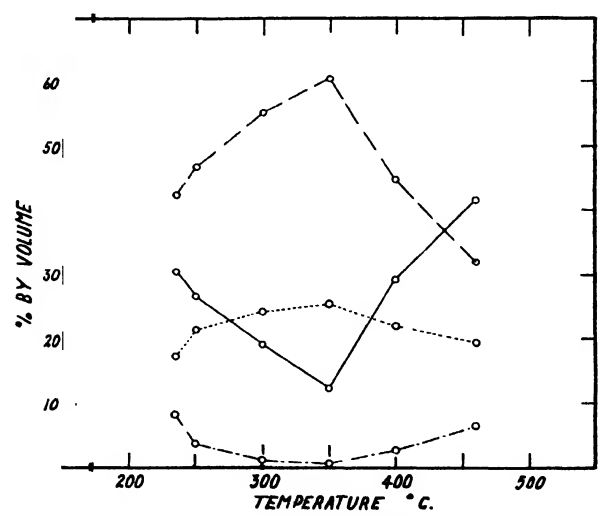

FIG. 1. *Results obtained with catalyst No. 53. Legend—composition of off-gases:* —— H_2, — — CH_4, ····· CO_2, —·—· CO.

composition at this temperature of maximum activity which would point to some change in the nature of the catalytic action. The gas composition as plotted in Fig. 2 for this catalyst shows graphically the extent of the change. The production of hydrogen increases rapidly and may be due to the entry of water into the reaction. Carbon was deposited on the catalyst at all temperatures and there was always an excess of carbon dioxide over that expected from the amount of methane present. The protective action of water with copper catalysts (3) which prevents secondary decompositions is not apparent here. The dehydrating power of chromium is almost completely suppressed in the presence of so much nickel.

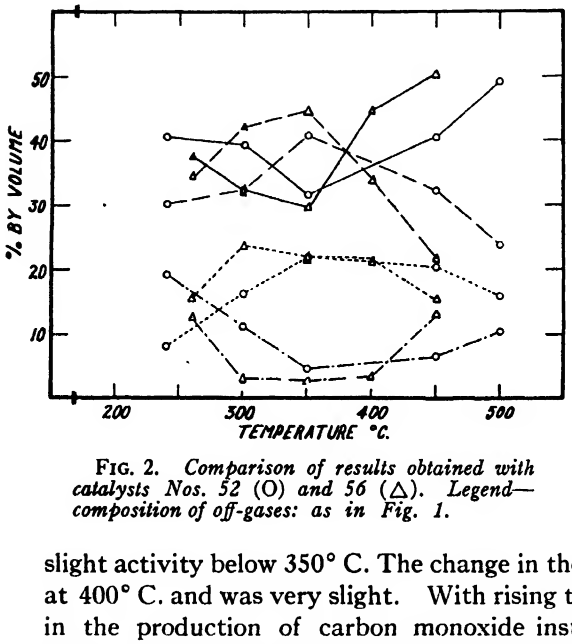

FIG. 2. *Comparison of results obtained with catalysts Nos. 52 (O) and 56 (△). Legend—composition of off-gases: as in Fig. 1.*

The results with catalyst No. 51 containing 86% of nickelic oxide were very similar to those obtained with catalyst No. 56. The change in the nature of the reaction occurred at 350° C. and carbon was deposited on the catalyst at all temperatures. This carbon was very reactive and easily removed by oxidation in a stream of oxygen at 300° C. The catalyst was again reduced with hydrogen and its activity examined. As shown in Table I this treatment altered the catalyst greatly resulting in very slight activity below 350° C. The change in the nature of the reaction occurred at 400° C. and was very slight. With rising temperature a maximum occurred in the production of carbon monoxide instead of a minimum, as shown by other catalysts, and the excess of carbon dioxide was even greater.

Catalysts Nos. 52 and 53 containing 80 and 66% of nickelic oxide respectively, were quite similar in their action to No. 56. The temperature at which the reaction altered was 350° C. Since these were both initial runs they seem to show an increase in this temperature with increasing chromium content. Both catalysts were heavily carbonized. They were less active at lower temperatures than those containing less chromium but were equally as good or better at higher temperatures. Figs. 1 and 2 show graphically the variation of gas composition with temperature and illustrate the change in the nature of the reaction in the region of 350° C.

Catalyst No. 54 with 50% of each oxide behaved quite differently from those preceding it. Although an abrupt temperature rise occurred when the alcohol reached the catalyst, the gas flow was small and intermittent at lower temperatures. Slight increases were noted in the percentage of methane and

oxides of carbon in the gas. It was also evident from the slight ethylene production that the dehydrating action of chromium was still very much in abeyance.

Catalyst No. 55, containing 40% of nickel and 60% of chromium oxides, was slightly less active than No. 54 but the gas analyses were very similar except at the higher temperatures. At 400° C. with a flow of 300 cc. per min. the decomposition of acetaldehyde was not as complex as usual nor was carbon formed to any great extent. Confirmation was clearly shown by the gas analysis. The yield of ethylene was surprisingly low and as before became smaller with rising temperature.

Catalyst No. 57, containing only 9% of nickelic oxide, was much less active than any in this series, producing a gas flow of only 20 cc. per min. at 325° C. and 40 cc. per min. at 400° C. Practically no carbon was formed on this catalyst. The dehydrating action of the chromium was becoming apparent although not to the extent that might be expected.

Another catalyst that should be considered with this series is No. 58, which contained 53% of nickelic oxide and was prepared with ammonium hydroxide as precipitant. The results are not tabulated since they correspond very closely to those obtained with catalyst No. 54. They illustrate, in this case, the fact that the properties of the catalyst are independent of the precipitating agent.

The dehydrating action of the chromium having been so thoroughly depressed, it was thought that the ultimate form of the chromic oxide might have been one of those mentioned by Sabatier (15) as essentially dehydrogenating. Catalyst No. 59 of pure precipitated chromic oxide partially verified this conclusion. As shown in Table II, at 340° C. the catalyst behaved about 25% as a dehydrating agent and 75% as a dehydrogenating agent. At 420° C. the gas analysis showed the reaction to be approximately 40% dehydration and 60% dehydrogenation. It was noted previously that the yield of ethylene decreased with rising temperature in the mixed catalysts but in this case the reverse is true. This would indicate that in the mixed catalysts described, the activity of the nickel increases more rapidly with temperature than does the activity of the chromium. The negligible production of carbon monoxide and carbon on this catalyst would suggest that the principal secondary reaction was the formation of liquid products and carbon dioxide from acetaldehyde.

Catalyst No. 60, of pure nickel, was examined in order to throw light on the total effect of the chromium in the mixed catalysts. The results of this series of runs are shown in Table II. The catalyst was carbonized and the gas analysis would suggest a fairly consistent and large decomposition of carbon monoxide to carbon dioxide. These results are very much as expected, showing an action at least 98% dehydrogenation. The higher the temperature the greater was the decomposition of the acetaldehyde. Due to secondary reactions there was a small excess of carbon dioxide over that to be expected from the decomposition of the carbon monoxide. It was noted that the catalyst was very sensitive and lost its activity rapidly. Subsequent runs to those shown in Table II gave gas flows of the order of one-half the tabulated values.

TABLE I

RESULTS OBTAINED ON PASSING ETHYL ALCOHOL VAPOR OVER VARIOUS MIXED CATALYSTS

Temp., °C.	Liquid flow, cc./min.	Composition of gas, % by vol.					Gas flow, cc./min.	Ratio liquid in/out
		CO_2	H_2	C_2H_4	CH_4	CO		
Catalyst No. 51; 86% nickelic oxide, after oxidation and reduction.								
450	0.75	17.1	58.5	nil	16.3	8.3	450	
410		15.7	44.4	0.7	27.1	12.1	300	
350		15.4	58.8	1.5	15.1	9.1	150	
Catalyst No. 52; 80% nickelic oxide.								
240	0.75	8.3	40.8	1.4	30.2	19.5	220	
300		16.4	39.4	0.5	32.4	11.3	600	1.5
350		22.0	31.8	0.5	40.9	4.7	800	
450		20.4	40.4	0.4	32.1	6.8	800	
500		16.0	49.3	0.4	23.9	10.4	800	4.8
Catalyst No. 53; 66% nickelic oxide.								
235	0.75	17.6	30.6	1.1	42.5	8.3	350	
250		21.6	26.8	0.9	46.8	3.9	500	
300		24.1	19.4	0.4	55.3	1.1	525	3.6
350		25.5	12.6	0.8	60.3	0.8	550	
400		22.0	29.2	0.4	44.7	2.9	600	
460		19.5	41.5	0.6	32.0	6.5	600	4.8
Catalyst No. 54; 50% nickelic oxide.								
280	0.75	9.1	39.4	1.1	33.2	17.2	150	
385		4.7	41.3	nil	31.2	22.9	320	
Catalyst No. 55; 40% nickelic oxide.								
350	0.75	2.5	69.5	1.1	20.8	6.0	120	
400		1.8	51.7	nil	24.1	22.3	320	
Catalyst No. 56; 97% nickelic oxide.								
First run								
235	0.75	16.6	39.0	0.7	33.9	9.9	400	
265		23.5	25.4	0.8	48.1	2.3	425	
300		24.4	17.8	0.4	57.0	0.6	600	
325		24.3	29.0	0.6	43.8	2.4	500	
350		23.1	30.9	0.6	43.1	2.2	450	2.4
Second run								
260	0.75	15.9	37.8	0.2	34.4	11.8	425	
300		23.7	32.2	0.8	42.1	3.0	600	
350		22.1	29.9	0.6	44.6	2.9	700	
400		21.5	44.8	0.2	33.9	3.4	525	
450		15.4	50.3	0.8	21.7	13.0	500	3.9
Catalyst No. 57; 9% nickelic oxide.								
400	0.75	3.6	64.7	4.6	14.0	13.2	40	

TABLE II

RESULTS OBTAINED ON PASSING ETHYL ALCOHOL VAPOR OVER SINGLE CATALYSTS

Temp., °C.	Liquid flow, cc./min.	Composition of gas, % by vol.					Gas flow, cc./min.	Ratio liquid in/out
		CO_2	H_2	C_2H_4	CH_4	CO		
Catalyst No. 59; pure precipitated chromic oxide.								
340	0.75	5.5	54.4	18.0	20.7	1.4	340	
420		8.7	51.0	30.4	8.2	nil	500	1.1
Catalyst No. 60; pure precipitated nickelic oxide.								
270	0.75	14.7	49.9	1.1	23.0	11.4	180	
285		21.2	52.8	1.0	20.1	4.8	375	
300		20.6	53.4	0.8	20.6	4.6	425	
350		21.2	42.8	0.6	29.2	6.2	500	
400		20.3	43.8	nil	30.5	5.5	500	2.2

The difference in catalytic activity of the simple and the mixed catalysts is well illustrated by a comparison of the results in Tables I and II. With the mixed catalysts, there were certain ranges of temperature in which a decided change in catalytic action and possibly catalyst surface occurred. The quantities of carbon dioxide and methane reach their maxima at this temperature on the mixed catalyst, while with the single catalysts they are at a minimum. The production of hydrogen reaches a minimum on the mixed catalysts and a maximum on the single catalysts in the same temperature range. This would indicate a more simple reaction on the single catalysts, involving to a large extent only decomposition of acetaldehyde subsequent to dehydrogenation.

The structure of a catalyst is conceded to have an important influence upon its activity (2). That these mixtures of catalysts are no exception to this rule is evident from the results with catalysts Nos. 46, 49 and 62, given in Table III. These catalysts all contained the same percentages of nickel and chromium but were prepared by different methods and received widely different treatments.

With catalyst No. 49, prepared by precipitation, it will be observed that the results are roughly similar to those previously described and shown in Table I. It should be noted however that there were relatively large amounts of carbon monoxide in these gases when compared with those of Table I, and that there was an increase in the ethylene production showing enhancement of the activity of the chromium. Less carbon monoxide was decomposed pointing to a reduction in the activity of the nickel. The catalyst lost some of its activity at 425° C. as was evident from the decreasing gas yield and increasing hydrogen concentration. A second run at 230° C. gave only 70 cc. of gas per min. The reduction in activity was probably due to the decrease in active surface by carbon deposition, as the gas analyses were the same with both runs at 230° C.

As might be expected, catalyst No. 46 was less active than the corresponding catalysts of Table I prepared from freshly precipitated hydroxides. The action started at 350° C. with a gas flow of 200 cc. per min. and dropped rapidly to a few cc. per min. At 450° C. only 55 cc. of gas was produced per min. Ethylene was present in appreciable quantities that increased with temperature. The catalyst was largely a dehydrogenation type and caused little secondary decomposition.

A portion of this same catalyst was reduced with pure alcohol instead of hydrogen. The catalyst was very much more active and contrasted strongly with the other sample. This was in accord with the results of Adkins and Lazier (1) which showed the influence of the reducing agent on the activity of the catalyst. Table III gives the results of two runs. In spite of carbon deposition during reduction and from the subsequent experimental runs, the gas flow was quite appreciable. The decomposition was comparatively simple and consisted of dehydrogenation followed by aldehyde decomposition. The percentage of ethylene was slightly less, showing that the effect of the alcohol reduction process is largely on the nickel.

Catalyst No. 62 of the ignited series was very inactive. The action of the chromium was more apparent in this catalyst and would indicate the greater sensitivity of nickel to harsh treatment.

TABLE III

THE EFFECT OF VARIOUS METHODS OF PREPARATION ON THE SAME CATALYST

Temp., °C.	Liquid flow, cc./min.	Composition of gas, % by vol.					Gas flow, cc./min.	Ratio liquid in/out
		CO_2	H_2	C_2H_4	CH_4	CO		
Catalyst No. 46; equimolar mixture of nickelous oxide and chromic oxide.								
350	0.75	6.9	78.7	2.4	8.5	3.5	slight	1.1
450		8.1	72.9	3.6	11.4	4.1	55	1.2
Same reduced with alcohol								
350	0.75	0.4	62.0	1.1	17.6	18.9	150	1.3
450		9.3	45.7	2.0	23.7	19.4	300	1.9
Catalyst No. 49; equimolar mixture of precipitated hydroxides of nickel and chromium.								
230	0.75	5.2	54.1	0.9	23.9	16.0	200	1.2
300		11.3	44.4	1.5	23.3	19.4	320	1.6
350		16.8	43.0	4.0	25.9	11.9	420	1.6
400		15.5	45.0	2.9	25.1	12.2	600	2.2
425		16.4	56.8	2.0	18.3	6.1	400	
Catalyst No. 62; equimolar mixture of nickel and chromium nitrates ignited at 1000° C.								
450	0.75	1.3	66.0	13.4	12.7	6.5	70	1.6

As shown in Table IV, catalyst No. 61 of the ignited series on supporting material was inactive even when compared with catalyst No. 62 which was

not supported. Catalyst No. 67 of the same type showed very similar results. The gas flow was very small with only a slight secondary decomposition of acetaldehyde. The activity of the chromium was again decreased.

The inactivity of ignited catalysts was not unexpected in view of the work of Taylor and Burns reported by Bancroft (4) and Gilfillan (8), but more than this, it was found with these mixed catalysts that the constituents were affected in widely varying degree. Supported catalysts frequently show an increased activity (7) but with the examples investigated the reverse was more nearly true. The unsupported mixed catalysts did not behave as though each metal were exerting its influence altogether independent of the other. The supported catalysts, however, retained only a portion of the activity of the individuals and on ignition lost all but a slight activity. Although very inactive they showed that two distinct primary reactions occurred, indicating that the catalyst was to some extent a mixture of two independent catalysts. The very common secondary reaction involving the formation of carbon dioxide and carbon from carbon monoxide did not occur on these catalysts.

Catalyst No. 63 using porcelain as a support for the precipitated oxides was comparatively inactive though not as unsatisfactory as the ignited series. The gas flows were 10, 20 and 25 cc. per min. at 350°, 450° and 500° C. respectively, and no carbon was deposited. The composition of the gas was independent of temperature and only one analysis is given in Table IV.

Catalyst No. 65, which was similar to No. 63 except that it was free from alkali, appeared slightly more active, and it will also be noticed that there was a great deal more ethylene produced or surviving in the presence of the excess alkali.

Attempts to activate these two catalysts met with no success. The small gas yields were contrary to expectation and might be explained on the basis of a smaller available surface. The gas analyses were so different from those obtained with catalysts Nos. 49 to 58 that the use of a supporting material must be considered as a fundamental alteration in the catalyst.

In the case of the catalysts supported on alumina, No. 68, prepared by ignition, was entirely inactive up to 400° C. and was not investigated further. Catalyst No. 69, prepared by precipitation, was much more active as shown in Table IV. Here again the support seemed to have altered the nature of the decomposition reactions appreciably.

The last in this series of mixed nickel and chromium oxides was No. 38 with the theoretical composition of nickel chromate. The first runs were made on a carbonized sample from previous work with alcohol mixtures. As shown in Table V action commenced at about 300° C. and the gas yield increased steadily with rising temperature.

A fresh sample of the same catalyst, after reduction with hydrogen for six hours, gave the results indicated. It will be noticed that on this more active catalyst, the ethylene production was decreased and the carbon dioxide was increased, the latter probably at the expense of the carbon monoxide. From the low hydrogen production it was evident that some hydrogenation of the carbon oxides occurred.

TABLE IV

EFFECT OF THE SUPPORTING MEDIUM ON THE ACTION OF CATALYSTS

Temp., °C.	Liquid flow, cc./min.	Gas composition, % by vol.					Gas flow, cc./min.	Ratio liquid in/out
		CO_2	H_2	C_2H_4	CH_4	CO		
Catalyst No. 61; equimolar solution of nickel and chromium nitrates ignited on asbestos.								
450	0.75	1.8	87.0	4.4	6.2	0.6	35	1.2
Catalyst No. 63; equimolar mixture of nickel and chromium hydroxides precipitated on porcelain with boiling KOH and drained.								
450	0.75	2.2	86.7	5.4	4.7	1.0	20	1.2
Catalyst No. 65; same as No. 63 with the excess alkali washed out.								
450	0.75	1.0	82.0	10.0	5.5	1.5	35	•
Catalyst No. 69; equimolar solution of nickel and chromium nitrates precipitated on fresh alumina gel with hot KOH.								
325	0.75	2.3	72 0	5.0	7.7	13.0	90	
400		2.8	64.1	7.7	15.0	10 3	210	

Another new sample of the catalyst was reduced with hydrogen for four hours. The solution used in this set of experiments was 34 mole per cent alcohol. The results as shown in Table V are very similar to those given with the previous sample for the equimolar solution and would suggest that the water is not entering into the reaction.

The same sample of catalyst was treated with oxygen at 300° C. to remove the carbon deposit. The carbon was very active, being converted rapidly to carbon dioxide at this temperature although it is probable that the actual temperature on the catalyst was greater than 300° C. After reduction with hydrogen, equimolar alcohol solution was passed over the catalyst. It will be noticed that the ethylene production was low, indicating that the effectiveness of the nickel had not been impaired relative to the chromium.

The action of prolonged reduction with hydrogen is of interest in the way the decomposition of carbon monoxide was affected. A used portion of catalyst No. 38 was oxidized and then reduced with hydrogen for 15 hr. at 300° C. This catalyst was less active than those reduced for a shorter time, as the catalyst showed no activity below 350° C. The gas composition shows the secondary decomposition of carbon monoxide to carbon dioxide to be comparatively slight.

A second portion of used catalyst was oxidized and then reduced with carbon monoxide. There was no formation of carbon on the catalyst during reduction. With alcohol solution the catalyst was very inactive with no reaction below 400° C.

A new sample of catalyst No. 38 was reduced at 295° C. with pure alcohol.

No carbonization of the catalyst occurred. This catalyst verified further the findings of Adkins and Lazier (1) on the influence of reducing agents. As shown in Table V, the catalyst reduced with alcohol was more active than that reduced with hydrogen and carbon did not form on the catalyst until the temperature was above 350° C. It has already been shown that catalyst No. 46 was also more active after reduction with alcohol than after hydrogen treatment, thus emphasizing the effect of the method of reduction on activity.

The temperature at which carbon deposition occurred corresponded approximately with the abrupt change in the nature of the reaction between 375° and

TABLE V

EFFECT OF VARIOUS TREATMENTS UPON CATALYST No. 38, NICKEL CHROMATE

Temp., °C.	Liquid flow, cc./min.	Gas composition, % by vol.					Gas flow, cc./min.	Ratio liquid in/out
		CO_2	H_2	C_2H_4	CH_4	CO		
Sample used in previous work.								
300	0.55	7.4	46.0	1.3	23.4	21.7	100	1.1
350		7.5	49.5	0.8	22.0	20 2	300	1.3
400		18 2	40.5	0.2	35.0	6.2	420	
Fresh sample.								
350	0.55	23.8	34.2	nil	38.3	3 7	420	3.5
450		23.2	32.7	0.8	42 2	1.1	280	4.4
Solution used containing 66% water.								
300	0.65	17.8	36 9	0.7	37.2	7.4	350	
350		20.5	27.8	0.4	47.9	5.4	400	
400		19.9	40.5	1.1	31.8	6.9	550	1 8
Above catalyst oxidized and reduced, re-run with equimolar alcohol and water.								
350	0.65	14 2	51.9	0.2	22.2	11.3	80	1.8
Oxidized and reduced with hydrogen for 15 hr.								
350	0.65	3 4	61.6	2.0	14.9	18.0	50	
400		7.1	47.5	0.7	25 4	19.3	270	3 2
Reduced with carbon monoxide.								
500	0.65	18.2	40.5	0 2	35.0	6.2	350	4.0
Reduced with ethyl alcohol.								
300	0.50	5.9	62.6	2.8	17.3	11.4	25	1.1
350		3.3	66.0	1.6	13.9	15.2	50	1.2
375		3.8	45.5	1.6	25.2	23.9	120	1.4
400		23.3	27.4	0.5	44.9	4.1	250	5.0
425		23.2	28.4	0.4	44.2	3.8	200	5.2

400° C. but there is no apparent relation to the action of the series 51 to 57. As in the results reported on other samples of catalyst No. 38, secondary decompositions occurred almost exclusively with carbon monoxide. A blank experiment carried out with active carbon in place of a metallic catalyst showed carbon to be relatively inactive. The abrupt change as shown was evidently a temperature effect.

Summary

A large amount of data has been secured which is rather difficult to interpret completely without analysis of the liquid products. Nevertheless certain conclusions may be stated. In mixed catalysts of the type described, the activity and the nature of the reactions generally depended largely on the method of preparation. The activity of the chromium was not evident unless the catalyst had undergone severe treatment. Nickel was much more sensitive to vigorous treatment than chromium.

A variety of reactions is possible and apparently the number and nature of the reactions that occurred were functions not only of the history of the catalyst, but also of the temperature. The nature of the reducing agent used in preparing the catalyst was of importance, at least in two cases investigated, in determining the course of secondary reactions.

Generally, the most active catalysts for the primary reaction also promoted the secondary reactions. A possible exception to this rule was found in nickel chromate which would have a distinctly different structure from catalysts of the mixed oxide type. In all catalysts the dehydrating power of chromium was largely suppressed by the presence of nickel.

References

1. Adkins, H. and Lazier, W. A. J. Am. Chem. Soc. 46: 2291-2305. 1924.
2. Adkins, H. and Lazier, W. A. J. Am. Chem. Soc. 48: 1671-1677. 1926.
3. Armstrong, E. F. and Hilditch, T. P. Proc. Roy. Soc. A. 97: 259-264. 1920.
4. Bancroft, W. D. J. Phys. Chem. 27: 801-941. 1923.
5. Boomer, E. H. and Morris, H. E. Can. J. Research, 2: 384-387. 1930.
6. Hoover, G. I. and Rideal, E. K. J. Am. Chem. Soc. 49: 104-115. 1927.
7. Gauger, A. W. and Taylor, H. S. J. Am. Chem. Soc. 45: 920-928. 1923.
8. Gilfillan, F. A. J. Am. Chem. Soc. 44: 1323-1333. 1922.
9. Lazier, W. A. and Adkins, H. J. Phys. Chem. 30: 895-898. 1926.
10. Lemoine, G. Compt. rend. 162: 702-708. 1916.
11. Mond, L., Langer, C. and Quincke, F. J. Chem. Soc. 57: 749-753. 1890.
12. Pease, R. N. and Taylor, H. S. J. Phys. Chem. 24: 241-265. 1920.
13. Rideal, E. K. and Taylor, H. S. Catalysis in theory and practice. Macmillan, 1926.
14. Russell, W. W. and Marschner, R. F. J. Phys. Chem. 34: 2554-2566. 1930.
15. Sabatier, P. Catalysis in organic chemistry. Van Nostrand, 1922.
16. Sabatier, P. and Mailhe, A. Compt. rend. 147: 106-110. 1908.
17. Sabatier, P. and Mailhe, A. Ann. chim. phys. (8) 20: 289-352. 1910.
18. Sabatier, P. and Senderens, J. B. Compt. rend. 136: 738-741. 1903.
19. Sabatier, P. and Senderens, J. B. Ann. chim. phys. (8) 4: 433-488. 1905.

STUDIES ON THE ACTION OF SULPHATES ON PORTLAND CEMENT

IV. THE ACTION OF SULPHATE SOLUTIONS ON MORTARS PREPARED FROM SOME BINARY AND TERNARY COMPOUNDS OF LIME, SILICA, ALUMINA AND IRON[1]

By T. Thorvaldson[2], D. Wolochow[3] and V. A. Vigfusson[4]

Abstract

A study was made of the action of solutions of the sulphates of magnesium, sodium and calcium on 1:10 mortar prisms. The prisms were made with standard sand and the following substances or mixtures of these: tricalcium silicate, β-dicalcium silicate, γ-dicalcium silicate, tricalcium aluminate, $5CaO.3Al_2O_3$, monocalcium aluminate, $3CaO.5Al_2O_3$, dicalcium ferrite and $4CaO.Al_2O_3.Fe_2O_3$. Some of the experiments dealt with mortars of richer mix (1:7½ and 1:5). The effect of the solutions was determined by measuring the linear expansion of the prisms and the tensile strength when the measurements of expansion were discontinued. A very pronounced difference was found to exist between the behavior of mortars made with mixtures rich in tricalcium silicate and those rich in β-dicalcium silicate. This observation is applied in the discussion of the resistance of the different types of hydraulic cements to the action of sulphate solutions.

Introduction

The scientific literature dealing with the disintegration of Portland cement when exposed to sulphate-bearing waters, such as sea water, mine waters, and the so-called "alkali" water, has reached very large proportions. Studies have been reported dealing with the action of these waters on both unhydrated and hydrated cement, on mortar and concrete specimens in the laboratory and in the field, and on concrete structures such as aqueducts, dams and docks. This literature contains reports of many apparently conflicting observations as to the permanence of well-made concrete, the relative harmfulness of the different sulphates or other salts which may be present in these waters, and the effects produced by varying the conditions of manufacture or curing of the concrete.

One of the obvious reasons for such inconsistencies is the fact that the most important basic material, the hydraulic cement, though conforming to certain specifications as to physical properties and falling within certain rather indefinite limits as to chemical composition, is in reality a highly variable material, considered from the standpoint of the actual chemical compounds present.

To illustrate this, one might consider the composition of, say, three commercial Portland cements which passed the standard specifications for all the physical requirements and gave on chemical analysis the results shown in Table I.

A comparison of these analyses with the range of values given by Eckel (4) for American Portland cements is given in Table II.

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TABLE I

COMPOSITION OF THREE PORTLAND CEMENTS

Cement No.	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	CaO %	MgO %	SO_3 %	Ign. loss %	Free CaO %
1	20.02	6.97	2.61	63.48	3.86	1.85	1.25	0.10
2	22.00	8.06	2.87	60.95	2.86	2.02	1.28	0.03
3	22.56	6.06	4.36	61.67	2.66	1.53	1.00	0.00

TABLE II

VARIATION IN COMPOSITION OF AMERICAN PORTLAND CEMENTS

	SiO_2 %	Al_2O_3 %	Fe_2O_3 %	CaO %	MgO %	SO_3 %
Eckel (4)	19.06–24.48	4.51–11.11	1.61–5.18	58.07–65.44	0.25–3.53	0.25–2.86
Cements Nos. 1, 2 and 3	20.02–22.56	6.06–8.06	2.61–4.36	60.97–63.48	2.66–3.86	1.53–2.02

It will be seen that the three Portland cements of Table I are of very uniform composition as compared with the American cements for which the analyses are given by Eckel. In no case does the percentage of lime, silica, alumina or iron oxide fall anywhere near the extreme values given, and the range between the highest and lowest value is in each case less than one-half of that given for commercial Portland cements of American manufacture. If one accepts in general the statement so often made, to the effect that commercial Portland cements are of very uniform chemical composition, then this would be doubly true of cements Nos. 1, 2, and 3 above.

When these three cements are made into mortar or concrete test pieces and exposed to fresh water, their behavior is very similar, but when exposed to water containing sulphates their behavior shows extreme differences. The chemical analyses as expressed above do not suggest a reason for this difference, but if one calculates the percentage of each of the chemical compounds present on the assumption that equilibrium had been attained during burning (the low percentage of free lime indicates that equilibrium had been attained) one is no longer astonished that there might be a difference in behavior when exposed to

TABLE III

CALCULATED COMPOUNDS PRESENT IN CEMENTS OF TABLE I

Cement No.	Percentage of compounds						
	Free lime	Tricalcium silicate	Dicalcium silicate	Tricalcium aluminate	$4CaO.Al_2O_3.Fe_2O_3$	Calcium sulphate	Magnesium oxide
1	0.10	50.0	19.7	14.05	7.94	3.15	3.86
2	0.03	16.8	50.4	16.51	8.73	3.43	2.86
3	0.00	28.3	43.3	8.67	13.30	2.60	2.66

aggressive waters. The results of such a calculation by the method of Colony (3) as modified by Bogue (2) are given in Table III.

It is thus seen that the percentages of the four most important compounds present vary greatly: tricalcium silicate from 16.8 to 50.0%, dicalcium silicate from 19.7 to 50.4%, tricalcium aluminate from 8.67 to 16.51%, $4CaO.Al_2O_3.Fe_2O_3$ from 7.94 to 13.30%. In view of the difference in the chemical properties of these compounds, one would expect great differences in the behavior of the different cements when attacked by corrosive solutions.

It is evident that a study of the reactions of sulphate solutions on the individual compounds present in hydraulic cements would be of primary importance in determining the material most resistant to the action of sulphate-bearing waters. The results of investigations of this kind have already been published, among these being the microscopic study by Shelton (13, 14, 15) in which he studied the action of sodium and magnesium sulphates on the substances, tricalcium silicate, β-dicalcium silicate and the four aluminates of calcium, both in the hydrated and the unhydrated form, and on a white Portland cement. Although such studies have given valuable information, their application to the disintegration of mortar or concrete in sulphate solutions presents difficulties.

Thorvaldson, Vigfusson and Larmour (18) studied the action of solutions of sodium, magnesium, and calcium sulphates on mortar bars made from pure tricalcium silicate, β-dicalcium silicate and tricalcium aluminate or mixtures of these mixed with standard sand (one part of silicate to five parts of sand by weight). The course of the action of the solution on the mortar bars was followed by means of accurate measurements of expansion. At the end of two years' exposure to solutions of sodium sulphate (2.2% and 8.1% of Na_2SO_4), under conditions which, in the case of similar specimens made from Portland cement, would have resulted in the initial indications of disintegration in from 5 to 10 days and complete failure in about one month, the specimens of mortar made from pure tricalcium silicate and β-dicalcium silicate showed no signs of being adversely affected. Exposure of mortar bars of the same composition to solutions of magnesium sulphate (1.9 and 6.8% of $MgSO_4$) caused slow expansion and ultimate disintegration, while similar bars, in which tricalcium aluminate to the extent of 25% of the amount of the silicate was present, expanded and disintegrated rapidly both in solutions of sodium sulphate and magnesium sulphate. A sand mortar made with a mixture of tricalcium silicate, β-dicalcium silicate, and tricalcium aluminate gave specimens which developed strength in a manner very similar to Portland cement and expanded and disintegrated very rapidly when exposed to solutions of the sulphates of sodium, magnesium and calcium, giving expansion-time curves which showed a somewhat more rapid expansion than similar bars made of Portland cement. The only marked difference observed was that the mortar made from the composite cement was not rendered as immune to the action of sulphates by curing in steam as similar specimens made from commercial Portland cement.

The experiments described in the present paper represent a more extensive

study of the same kind, including not only mortar specimens made with the compounds present in Portland cement, but also those present in high alumina and iron ore cements.

The value of expansion measurements as a means of following the progress of the disintegration of mortar and concrete specimens made from Portland cement and exposed to sulphate solutions has already been discussed by the authors (19). Not only is the expansion a measure of the stress which would be developed if the material was not free to expand, but it is also possible to predict from such measurements the loss in strength of the test pieces (10, 17). The application of the method to the prediction of loss of strength in the case of mortars prepared from cements, the composition of which does not fall within the limits for Portland cement, is not so certain. In the present series of experiments however, work is carried on under conditions which, for corresponding mixes of ordinary Portland cements, produce visible effects on the specimens in the course of a week or two and complete disintegration as a rule within one month. It is, therefore, evident that expansion measurements extending over three years' exposure combined with observations as to any visible physical effects, and, in many cases, determinations of tensile strength of the specimens, will in any case give fairly reliable evidence as to the resistance of the material being studied.

Preparation of Cement Substances*

The cement compounds were with slight modifications prepared according to the methods outlined by Rankin (11, 12), Bates and Klein (1), Lerch and Bogue (8) and Hansen, Brownmiller and Bogue (7). The raw materials used in the preparation were as follows: (1) White marble which on ignition gave a residue of 56.27% and which showed only traces of impurities present. (2) Flint which contained 98.45% of silica. (3) Hydrated alumina, a very pure sample containing 65.28% of alumina. (4) Ferric oxide, a c.p. sample of high purity. With the exception of the flint, which was much more finely ground, these materials were pulverized to pass a 200-mesh sieve, and were mixed very thoroughly in the required proportions before firing. Sufficient water was added to the mixed materials (in some cases coal oil was used) to enable them to be molded into hollow cylinders which were dried and then fired either in a compressed air-oil, or in a gas-fired, furnace until well sintered, the temperature being determined by a Leeds and Northrup optical pyrometer. The sintered material was then broken up, reground to pass a 200-mesh sieve and refired, this process being repeated as often as was necessary to obtain complete combination as shown by the absence of free lime, and by a uniform refractive index. A complete chemical analysis of the product was usually made as a further check before the final firing.

In the preparation of tricalcium silicate the initial mixture was of the composition $2CaO:SiO_2$. This was fired at about 1550°C. until the product contained no free lime and after dusting was composed almost entirely of

**The authors wish to acknowledge the very capable and valuable collaboration of G. R. Shelton and N. H. Grace in the preparation and analyses of these substances.*

γ-dicalcium silicate. The extra mole of lime required to form tricalcium silicate was then added in three portions of 0.5, 0.25, and 0.25 mole, with intermediate firing at 1500° to 1580°C. After a final regrinding and firing for six hours at this temperature, the microscopic examination indicated a uniform product free from particles of lime, having a refractive index of 1.715. White's test (20) was negative and a determination of lime by the method of Emley as modified by Lerch and Bogue (9) also gave a negative result.

The preparation of γ-dicalcium silicate was similar to the first step in the production of tricalcium silicate, the fired cylinder being cooled very slowly to favor the inversion from the β- to the γ-form. Microscopic examination showed only γ-dicalcium silicate.

In the preparation of β-dicalcium silicate the main problem is the prevention of the inversion of the substance to the γ-form. Chromic oxide (0.5%) was added to the mix and the cylinder was quenched in cold water Even with this treatment the final sample contained small amounts of the γ-form.

The sample of tricalcium aluminate was prepared by repeated firing of the cylinder at a temperature of 1300° to 1400°C. It was found that addition of water to the finely ground material between firings hastened combination, probably on account of the hydration of any high-burned lime present in the material. Microscopic examination showed the presence of a very small amount of $5CaO.3Al_2O_3$ in the final sample. The other three aluminates were prepared by firing the required mixtures of calcium carbonate and hydrated alumina at temperatures somewhat below the melting points of the respective aluminates. None of the products contained any free lime. The monocalcium aluminate contained a slight trace of $5CaO.3Al_2O_3$ while the sample of $3CaO.5Al_2O_3$ was entirely homogeneous. The sample of dicalcium ferrite was fired for two periods of about two hours each at a temperature of 1150° to 1200°C. After the second firing the material was homogeneous. The compound $4CaO.Al_2O_3.Fe_2O_3$ was fired for two three-hour periods at about 1250°C. The final product was homogeneous and had the optical properties given by Hansen, Brownmiller and Bogue (7).

Preparation and Curing of Mortar Specimens

A summary of the composition of the "cements" used in the main series of experiments is presented in Table IV. The specimens used for the expansion measurements were mortar bars made from the single or mixed compounds and standard Ottawa sand (20-30 mesh), distilled water being used for obtaining a plastic mix. The proportion was, unless otherwise specified, 1 part of the compound or mixture of compounds to 10 parts of standard sand by weight. This is an extremely lean mix, so that the specimens were very permeable to the solutions and very rapid action was therefore to be expected. The specimens were made in collapsible steel frames so as to give rectangular prisms measuring 1.55 by 1.55 by 10 cm. A smooth end was obtained by means of a thin layer of neat cement made from the cementing substance used in each case. The molds were stored in the damp closet until the bars were strong enough to be removed without serious accidental breakage. Those containing

TABLE IV

COMPOSITION OF "CEMENTS" USED IN MAIN SERIES OF EXPERIMENTS (SERIES 101. 1:10 MORTARS)

Serial letter	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	ZZ
Batch No.	151	152	176	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	177	178	179	180	181	182	183	184	185
$3CaO.SiO_2$, %	100						80	80	80	80	80	80							50	40	40	40	40	40	40	40	40
β-$2CaO.SiO_2$, %		100											80	80	80	80	80	80	50	40	40	40	40	40	40	40	40
γ-$2CaO.SiO_2$, %			100																								
$3CaO.Al_2O_3$, %							20						20							20						10	6.6
$5CaO.3Al_2O_3$, %				100				20						20							20					10	6.6
$CaO.Al_2O_3$, %					100				20						20							20					
$3CaO.5Al_2O_3$, %						100				20						20							20				
$2CaO.Fe_2O_3$, %											20						20							20			6.6
$4CaO.Al_2O_3.Fe_2O_3$, %												20						20							20		
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
CaO, %	73.7	65.1	65.1	47.8	35.5	24.8	71.4	68.5	66.0	63.9	67.2	68.2	64.6	61.7	59.2	57.1	60.4	61.4	69.4	68.0	65.1	62.6	60.5	63.8	64.8	66.5	65.6
SiO_2, %	26.3	34.9	34.9	—	—	—	21.1	21.1	21.1	21.1	21.1	21.1	27.9	27.9	27.9	27.9	27.9	27.9	30.6	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5
Al_2O_3, %	—	—	—	52.2	64.5	75.2	7.5	10.4	12.9	15.0	—	4.2	7.5	10.4	12.9	15.0	—	4.2	—	7.5	10.4	12.9	15.0	—	4.2	9.0	6.0
Fe_2O_3, %	—	—	—	—	—	—	—	—	—	—	11.7	6.5	—	—	—	—	11.7	6.5	—	—	—	—	—	11.7	6.5	—	3.9
Total, %	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

80% or more of tricalcium silicate, or the three aluminates possessing marked hydraulic properties ($5CaO.3Al_2O_3$, $CaO.Al_2O_3$, $3CaO.5Al_2O_3$) were removed in 20 days. They were then all in excellent condition except the batches containing dicalcium ferrite (161) and $4CaO.Al_2O_3.Fe_2O_3$ (162), which were weak. At that time the bars containing a large amount of β-dicalcium silicate were rather weak, so they were left in the molds for another two weeks. The bars containing the mixture of β-dicalcium silicate and dicalcium ferrite or $4CaO.Al_2O_3.Fe_2O_3$ (Batches 167-168) were still soft and even after a total curing time of seven weeks many broke on being removed from the molds. The bars made from γ-dicalcium silicate did not appear to set for several weeks but at the end of 10 weeks it was possible to remove them from the molds without excessive breakage. After removal from the molds the bars were placed in distilled water until eight weeks old, when they were exposed to the solutions. The only bars treated differently were those containing γ-dicalcium silicate and these were cured in water until five months old, and then exposed to the solutions.

Experimental Procedure

Three sulphate solutions were used in these experiments, namely 0.15 *M* Na_2SO_4 (2.1% by weight); 0.15*M* $MgSO_4$ (1.8% by weight); and a saturated solution of calcium sulphate. A few experiments were made with 0.5 *M* solutions of the first two salts. The solutions of sodium and magnesium sulphates were prepared from recrystallized salts and contained only traces of impurities. The solution of calcium sulphate was prepared from a pure sample of ground gypsum, a large excess of the gypsum powder being added to each jar of saturated solution and the solution thoroughly stirred from time to time. The bars were exposed to the sulphate solutions in pint glass jars which were tightly sealed except when measurements were being made. Duplicate bars were immersed in each jar containing 450 cc. of the solution.

Measurements of the lengths of the bars were made from time to time with a micrometer head set in a steel frame. The micrometer head was graduated to read to 0.01 mm. so that the lengths of the 10 cm. bars were determined to 1 part in 10,000 (0.01%). All the experimental work was carried on in constant temperature rooms at 21°C. (70°F.) The temperature of the solutions in the jars rarely varied more than $\pm$ 0.1°C. from this mean.

Experimental Results

I. Mortars of the Calcium Silicates and Sand

The solutions in which the mortar bars were immersed are indicated in Table V. The bars were composed of 1 part by weight of the silicate indicated and 10 parts of standard sand, 20-30 mesh. Each solution is always represented by the same numeral while the letter refers to the composition of the cementing substance as given in Table IV. The combined number and letter therefore identify the composition of the bar and of the solution in which it was immersed and will be used in the text to refer to the corresponding expansion-time curves which are all labelled in this manner.

TABLE V

EXPOSURE OF 1:10 MORTARS MADE FROM THE PURE SILICATES OF CALCIUM

Silicate	Solution				
	Na_2SO_4		$MgSO_4$		$CaSO_4$
	0.15*M*	0.50*M*	0.15*M*	0.50*M*	Saturated at 21° C.
$3CaO.SiO_2$	1A	3A	2A	4A	21A
β-$2CaO.SiO_2$	1B	3B	2B	4B	21B
γ-$2CaO.SiO_2$	1C	—	2C	—	21C
{50% $3CaO.SiO_2$ 50% β-$2CaO.SiO_2$	1S	—	2S	—	21S

Expansion Curves for the Silicate Mortars in Solutions of Magnesium Sulphate

Fig. 1 shows the expansion-time curves for the experiments outlined in Table V. It will be seen that the 1:10 mortar bars made of tricalcium silicate, β-dicalcium silicate, γ-dicalcium silicate and the mixture of 50% of tricalcium silicate and 50% of β-dicalcium silicate all expand in solutions of magnesium sulphate (Curves 2A, 2B, 2C, 2S) and that the rate of expansion increases with the concentration of the sulphate solution (Curves 2A and 4A, 2B and 4B).

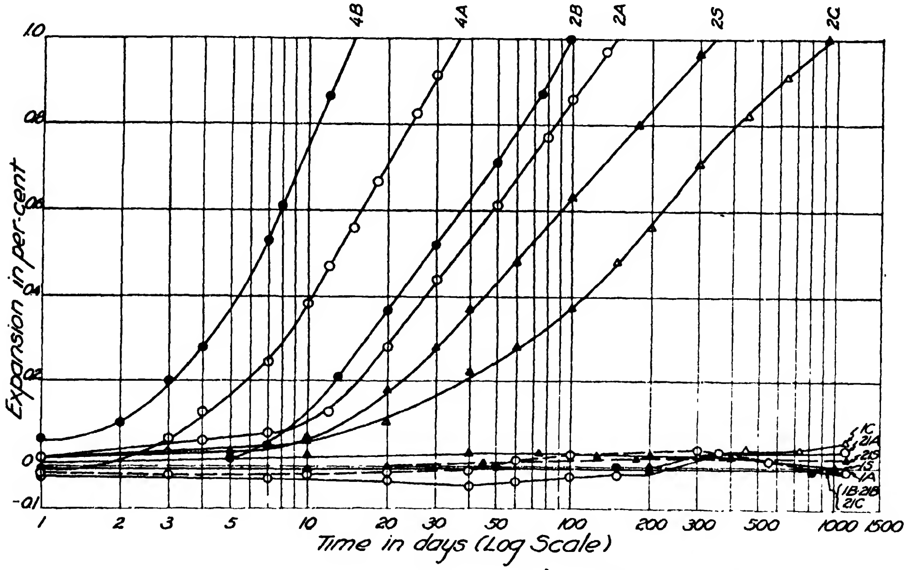

FIG. 1. *Expansion of 1:10 sand mortars made with the calcium silicates as the cement, in 0.15M and 0.50M $MgSO_4$, 0.15M Na_2SO_4 and a saturated solution of $CaSO_4$.*
Legend: A = $3CaO.SiO_2$; B = β-$2CaO.SiO_2$; C = γ-$2CaO.SiO_2$; S = 50% $3CaO.SiO_2$ + 50% β-$2CaO.SiO_2$. 1 = 0.15M Na_2SO_4; 2 = 0.15M $MgSO_4$; 4 = 0.50M $MgSO_4$; 21 = Saturated $CaSO_4$.

In each case the rate of expansion is more rapid for the bars containing β-dicalcium silicate than for bars containing tricalcium silicate cured for the

same length of time (56 days) before exposure (Curves 2A and 2B, 4A and 4B). The very slow expansion of the bars containing γ-dicalcium silicate (Curve 2C) may be in part due to the long curing period of these bars (five months) and in part to the small amount of lime liberated by this compound. It is interesting to note that the bars containing tricalcium silicate and dicalcium silicate in equal proportions (Curves 2S) have a slower rate of expansion than the bars made with either of these silicates alone (Curves 2A and 2B). A comparison of the expansion of the 1:10 silicate mortars with that of similar mortar bars made from Portland cement No. 1 of Table I is shown in Table VI.

TABLE VI

COMPARISON OF TIME REQUIRED FOR LINEAR EXPANSION OF 0.5 AND 1%. 1:10 SAND MORTAR BARS IN 0.15M $MgSO_4$ AT 21° C.

Expansion, %	Cement				
	P.C. No. 1	β-2CaO SiO_2	3CaO.SiO_2	50% β-2CaO.SiO_2 50% 3CaO SiO_2	γ-2CaO.SiO_2
	Serial No.				
	2	2B	2A	2S	2C
	Time in days				
0.5	13	28	35	65	160
1.0	20	100	145	320	900

As the richness of mix is increased, this difference between the rates of expansion of bars made with the pure silicates and of bars made with Portland cement increases rapidly. Thus for 1:5 bars of tricalcium silicate, cured 2 months before exposure to the solution of magnesium sulphate, an expansion of 1% was attained in 1500 days in a 0.16 M solution of magnesium sulphate as against 48 days for similarly treated 1:5 bars of Portland cement No. 1 (Table I). Similar results were obtained when 1:10 and 1:5 bars of β-dicalcium silicate were compared with Portland cement bars of corresponding mix.

Tensile Strength of Silicate Bars Exposed to Magnesium Sulphate

On account of the large amount of material necessary for a systematic determination of loss of tensile or compressive strength during exposure to sulphate solutions, data of this kind are not available. Some of the bars exposed to the solutions were, however, tested for tensile strength in a special clamp which was used in the ordinary briquet testing machine. The determinations were usually made at the time when the expansion measurements were discontinued. The data obtained are shown in Table VII, the tensions being calculated in lb. per sq. in.

Although tension tests of the 1:10 silicate-sand mortars at the time of exposure to the sulphate solutions are not available, other data obtained in this laboratory make it possible to assume that the tensile strength of the tricalcium silicate bars (2A and 4A) would have been considerably below the

TABLE VII

TENSILE STRENGTH OF 1:10 SILICATE BARS AFTER EXPOSURE TO MAGNESIUM SULPHATE SOLUTIONS

Serial No.	Silicate	Solution	Length of exposure, days	Expansion, %	Tension, lb. per sq. in. after exposure to:	
					Solution	Water
4A	$3CaO.SiO_2$	$0.50M$ $MgSO_4$	210	>2.2	75	
2A	$3CaO.SiO_2$	$0.15M$ $MgSO_4$	200	1.1	65	
2B	β-$2CaO.SiO_2$	$0.15M$ $MgSO_4$	200	1.3	38	
2S	50% $3CaO.SiO_2$ 50% β-$2CaO.SiO_2$	$0.15M$ $MgSO_4$	325	1.05	42	73 (3 years)
2C	γ-$2CaO.SiO_2$	$0.15M$ $MgSO_4$	1000	1.0	44	10 (3 years)
	Portland cement*	$0.50M$ $MgSO_4$	13	1.0	30	90 (8 weeks)
	Portland cement*	$0.15M$ $MgSO_4$	20	1.0	12	90 (8 weeks)

**Cement No. 1, Table 1.*

value obtained for similar bars of cement No. 1, *i.e.*, 90 lb. per sq. in. The tensile strength of the bars made with β-dicalcium silicate (2B) would have been somewhat more than half of this value at eight weeks. The value for the mixture of the two silicates after three years' exposure to water (2S), 73 lb. per sq. in., may be somewhat low (only one break). It will be seen that the reduction in the tensile strength of the silicate bars after exposure to the sulphate solutions for long periods of time was remarkably small as compared with the rapid loss in strength of similar Portland cement bars. In the case of γ-dicalcium silicate (2C) there is a remarkable increase in strength, probably due to the speeding up of the hydration of this inert silicate by the sulphate.

Similar results were obtained with richer mixes of the silicate, namely, with bars containing one part by weight of the silicate to five parts of standard sand exposed to solutions of magnesium sulphate for longer periods. Bars of tricalcium silicate (1:5) were cured in the damp closet for two months before exposure to a 0.16 M solution of magnesium sulphate. After immersion in the solution for 50 months they had expanded 1.03% of their length and had a tensile strength of 184 lb. per sq. in., as compared with 198 lb. per sq. in for bars immersed in distilled water. At the end of 80 months' exposure the expansion was only 1.07% and the tension 175 lb. as against 192 lb. per sq. in. obtained for the water blank at that time. In the case of bars of β-dicalcium silicate (1:5) immersed in a 0.16 M solution of magnesium sulphate, a linear expansion of 3.8% was attained in 50 months and the tension was 55 lb. per sq. in. as compared with 187 lb. per sq. in. for similar bars immersed in distilled water. Thus very great expansion takes place before the strength of mortar made of the calcium silicates falls to a low value. In the case of 1:5 bars made with Portland cements immersed in 0.15 M $MgSO_4$, an expansion of 0.25% usually means a loss of 60 to 70% of the tensile strength while an expansion of 1% usually causes a reduction of about 90% of the original strength.

Expansion Curves for the Silicate Mortars in Solutions of Sodium and Calcium Sulphates

The results obtained on exposure of the 1:10 silicate bars to solutions of sodium sulphate and calcium sulphate (Fig. 1. Curves 1A, 1B, 1C, 1S, 21A, 21B, 21C, 21S, and Table VIII) show a striking contrast to the results of exposures to solutions of magnesium sulphate. Considering the expansion measurements at the end of three years' exposure there is no evidence of any appreciable deleterious action, the expansion being approximately the same as that obtained on exposure to distilled water.

On exposure of the silicate bars to the solutions there appears to be in general a slight tendency to contract. This, however, is soon followed by a slight gradual expansion, which usually reaches a maximum after an exposure of about one year, when a second period of very slow contraction of the bars sets in. The only exceptions to this are the bars of γ-dicalcium silicate in 0.15 M Na_2SO_4 (Curve 1C) which expanded to a maximum of 0.06% elongation at the end of the three-year period and the bars containing tricalcium silicate immersed in saturated calcium sulphate (Curves 21A and 21S) which are still near the maximum expansion of 0.04% and 0.02%, respectively, at the end of three years. For a summary of the data see Table VIII.

TABLE VIII

EXPANSION OF SILICATE MORTARS (1:10) IN SOLUTIONS OF SODIUM AND CALCIUM SULPHATES

Serial No.	Silicate	Solution	Minimum expansion		Maximum expansion		Expansion after 3 years' exposure, %
			%	Time	%	Time	
3A	$3CaO.SiO_2$	0.50 M Na_2SO_4	−0.01	10 da.	0.04	1.0 yr.	0.02
1A	$3CaO.SiO_2$	0.15 M Na_2SO_4	−0.02	{10 da. 3 yr.	0.04	1.0 yr.	−0.02
21A	$3CaO.SiO_2$	Sat'd $CaSO_4$	−0.04	50 da.	0.04	1.0 yr.	0.04
3B	β-$2CaO.SiO_2$	0.50 M Na_2SO_4	−0.01	1 da.	0.05	1.5 yr.	0.03
1B	β-$2CaO.SiO_2$	0.15 M Na_2SO_4	−0.02	3 yr.	0.00	—	−0.02
21B	β-$2CaO.SiO_2$	Sat'd $CaSO_4$	−0.02	3 yr.	0.00	—	−0.02
1C	γ-$2CaO.SiO_2$	0.15 M Na_2SO_4	0.00	—	0.06	3 yr.	0.06
21C	γ-$2CaO.SiO_2$	Sat'd $CaSO_4$	−0.02	50 da.	0.01	1 yr.	−0.01
1S	{50% $3CaO.SiO_2$	0.15 M Na_2SO_4	0.00	—	0.02	40 da.	0.02
21S	50% β-$2CaO.SiO_2$	Sat'd $CaSO_4$	0.00	—	0.02	1 yr.	0.02

These results are in agreement with those obtained by Thorvaldson, Vigfusson and Larmour (18) for 1:5 bars of tricalcium silicate and β-dicalcium silicate. It was thought that leaner mixes might show expansion, but it is now apparent that mortars of the pure silicates are extremely resistant to the action of sodium sulphate and calcium sulphate even where the salt solution has very free access to the particles of hydrated silicate as is certain to be the case with the 1:10 mortar bars. The possibility of expansion and deterioration taking place after the three-year period of exposure is not excluded and would seem possible or even probable since the solutions have, as indicated later, affected the process of hydrolysis of the silicates in the bars and a microscopic examination of the mortar showed the presence of large quantities of crystals of

gypsum. There is also still the possibility that the strength of the specimens may have been affected without any expansion taking place. No determinations of the tension of the 1:10 mortars after exposure to the solutions of sodium or calcium sulphate have been made. Such data are however available for 1:5 mortar bars of the same cross section (1.55×1.55 cm.) made of tricalcium silicate and β-dicalcium silicate after exposure for four years to solutions of sodium sulphate. The results were as shown in Table IX.

TABLE IX

A COMPARISON OF THE TENSILE STRENGTHS OF BARS OF CEMENT (CROSS SECTION 1.55×1.55 CM.), AFTER THE EXPOSURES INDICATED

Specification of bar	Tensile strength in lb. per sq. in. after 4 yr. exposure to:		
	Sodium sulphate solutions		Distilled water
	0.6 M	0.16 M	
1:5 Tricalcium silicate	232		198
1:5 β-Dicalcium silicate	256	251	187

Thus the silicate mortar exposed to solutions of sodium sulphate not only showed no appreciable expansion at the end of four years but also had a markedly higher tensile strength than specimens kept the same length of time in distilled water. This observation is not unexpected since specimens of Portland cement mortar exposed to sulphate solutions at first increase in strength more rapidly than similar specimens kept in water (17), and the liberation of lime also proceeds more rapidly and further when Portland cement is exposed to solutions of sodium sulphate than when exposed to pure water (16). As tricalcium aluminate does not liberate lime on hydration, it appears that the sulphate causes a more rapid and greater hydrolysis of the silicates.

These results with the silicate mortars also show a striking contrast to the behavior of similar mortar bars made with a typical Portland cement. Bars of 1:10 mix made with cement No. 1 (Table I) similarly treated and exposed to 0.15 M Na_2SO_4 showed a linear expansion of 0.04% in eight days and 1% in 15 days. The tension of the bars when exposed to the solution (at eight weeks) was 90 lb. per sq. in. but after 15 days' exposure the tension was less than 5 lb. per sq. in. The corresponding 1:5 mortar bars in 0.50 M Na_2SO_4 expanded to 0.04% in 14 days and to 1% in 37 days. The initial tension was 270 lb. per sq. in. and the tension at an expansion of 1%, 30 lb. per sq. in. Comparative tests with some 50 commercial Portland cements have shown that the mortar made from this particular cement has a resistance to sulphate solutions well above the average for commercial Portland cements.

II. Mortars of the Calcium Aluminates and Sand

Table X shows a summary of the exposures of calcium aluminate mortars (1 part aluminate and 10 parts standard sand by weight) to sulphate solutions. Tricalcium aluminate could not be included in this series of experiments since

the test pieces made of this aluminate and sand soon fall to pieces when exposed to pure water.

TABLE X

EXPOSURE OF 1:10 MORTARS MADE FROM CALCIUM ALUMINATES

Material in bars	Solution				
	$MgSO_4$		Na_2SO_4		$CaSO_4$
	0.15 *M*	0.50 *M*	0.15 *M*	0.50 *M*	Saturated at 21° C.
$5CaO.3Al_2O_3$	2D	4D	1D	3D	21D
$CaO.Al_2O_3$	2E	4E	1E	3E	21E
$3CaO.5Al_2O_3$	2F	4F	1F	3F	21F

A similar series of exposures was made with a mortar containing one part of the aluminate to seven and a half parts of standard sand by weight.

The measurement of the expansion of mortar bars of the kind used in this investigation does not appear to be as suitable for the study of the action of sulphates on the pure aluminates as on the silicates. The expansion is often erratic, especially at the beginning of the experiment, this probably being due to the tendency toward localized action of the sulphate and the low stability of the ends made of aluminate paste. This erratic behavior applies more particularly in the case of exposures of these mortars to concentrated solutions of sodium sulphate. The action of sodium sulphate, even in such a lean mortar as 1:10, tends mainly to progress gradually from the surface inwards while the layer of disintegrated material sloughs off as the action advances, leaving an apparently unaffected hard core. The expansion in solutions of magnesium sulphate and calcium sulphate is much more regular. Fig. 2 shows the curves for the expansion of the 1:10 bars in 0.15 and 0.50 *M* solutions of magnesium sulphate, 0.15 *M* solution of sodium sulphate and in a saturated solution of calcium sulphate. It is evident that the rate of expansion decreases with decreasing percentage of lime in the alumin-

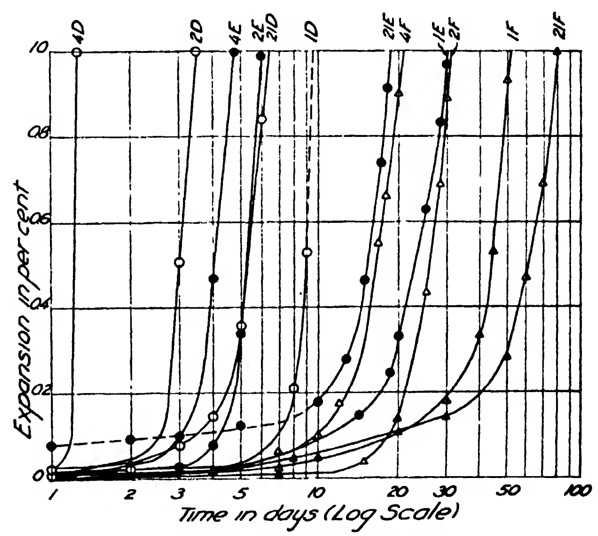

FIG. 2. *Expansion of 1:10 sand mortars made with the calcium aluminates as the cement, in 0.50 M and 0.15 M $MgSO_4$, 0.15 M Na_2SO_4 and a saturated solution of $CaSO_4$. Legend: D = 5 CaO. 3Al_2O_3; E = CaO Al_2O_3; F = 3CaO.5Al_2O_3; 1 = 0.15 M Na_2SO_4; 2 = 0.15 M $MgSO_4$; 4 = 0.50 M $MgSO_4$; 21 = Saturated $CaSO_4$.*

ate, being highest in each case for the bars containing the aluminate $5CaO.3Al_2O_3$ (Curves D) and lowest for the bars containing $3CaO.5Al_2O_3$ (Curves F). The rate also increases with the concentration of the sulphate solution.

While the bars exposed to solutions of sodium sulphate crumbled and shed their surface layer continuously (in some cases till the cross section of the bar was about one-quarter of the original), the hard core sometimes expanded 2% or more before it fell to pieces. In solutions of magnesium sulphate the bars did not crumble but kept their shape and remained fairly firm until they had reached a very high expansion. In the case of the 1:10 bars of $5CaO.3Al_2O_3$ in 0.50 *M* $MgSO_4$ (Curve 4D) the maximum expansion was as much as 10% of the original length, this elongation being attained in the short space of three days' exposure to the solution. The corresponding maximum expansion for the bars of monocalcium aluminate ($CaO.Al_2O_3$) was 5.5% in 7 days (Curve 4E) and for the $3CaO.5Al_2O_3$ bars 3% in 35 days (Curve 4F). In the saturated solution of calcium sulphate the corresponding maximum expansions were 5% in 25 days (Curve 21D), 3.5% in 35 days (Curve 21E) and 2% in 120 days (Curve 21F). The expansion and loss in strength for the aluminate mortars is very rapid compared with that of the mortars made of the calcium silicates.

The aluminate bars of 1:7½ mix were cured in the damp closet for seven days before they were exposed to the solutions. The data obtained are shown in Fig. 3. The most striking difference between the results of the two series is the marked increase in resistance which some of the richer mixes exhibit. The leaner mix (1:10) contained 9.11% while the richer mix (1:7½) contained 11.76% of the aluminates, an increase of 29%. In the case of the mortars

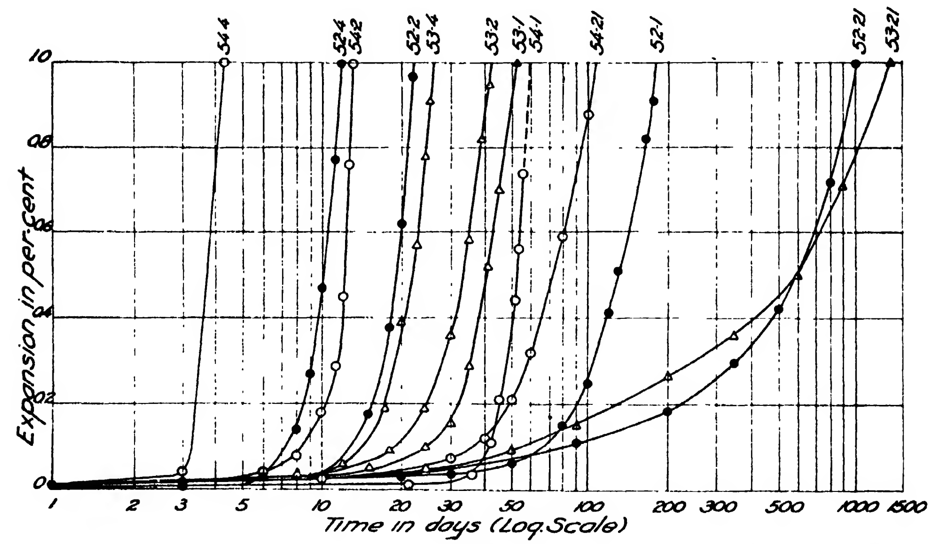

FIG. 3. *Expansion of 1:7½ sand mortars made with the calcium aluminates as the cement, in 0.5 M and 0.15 M $MgSO_4$, 0.15 M Na_2SO_4 and a saturated solution of $CaSO_4$.*
Legend: 54 = $5CaO.3Al_2O_3$; 52 = $CaO.Al_2O_3$; 53 = $3CaO.5Al_2O_3$. 1 = 0.15M Na_2SO_4; 2 = 0.15M $MgSO_4$; 4 = 0.50M $MgSO_4$; 21 = Saturated $CaSO_4$.

containing $3CaO.5Al_2O_3$, excepting those immersed in saturated calcium sulphate, there is very little increase in the time required for the 1:7½ bars to reach a given expansion as compared with the 1:10 bars (Fig. 2, Curves 1F, 2F and 4F; Fig. 3, Curves 53-1, 53-2, and 53-4). In the case of the mortars containing monocalcium aluminate the time required for the 1:7½ bars to expand 1% is from 3 times (when exposed to 0.5 *M* $MgSO_4$, Curves 4E and 52-4) to 50 times (when exposed to saturated $CaSO_4$, Curves 21E and 52-21) as long as the time required for the 1:10 bars. There are also corresponding increases in the time required for an expansion of 1% ranging from 2 to 18 times in the case of the bars containing $5CaO.3Al_2O_3$ (*cf.* Curves 1D and 54-1; 2D and 54-2; 4D and 54-4; 21D and 54-21). It has been found that commercial high alumina cements also show large differences between the resistance of lean and moderately rich mortars to the action of sulphate solutions.

The proportionally greater decrease in the expansion of the 1:7½ bars of monocalcium aluminate causes the rate of expansion in 0.15 *M* Na_2SO_4 and in saturated $CaSO_4$ to drop below that of the 1:7½ bars of either $5CaO.3Al_2O_3$ or $3CaO.5Al_2O_3$. The order of the rates of expansion in solutions of magnesium sulphate still remains the same as for the 1:10 bars.

III. The Effect of the Addition of the Calcium Aluminates, Dicalcium Ferrite and the Compound $4CaO.Al_2O_3,Fe_2O_3$ to Tricalcium Silicate and β-Dicalcium Silicate

Table XI gives a summary of the composition of the "cement" in the 1:10 sand mortar bars and the solutions in which they were immersed.

TABLE XI

SUMMARY OF EXPOSURES*

Solutions	80% $3CaO.SiO_2$+20% of:					
	C_3A	C_5A_3	CA	C_3A_5	C_2F	C_4AF
0.15 *M* $MgSO_4$	2G	2H	2I	2J	2K	2L
0.15 *M* Na_2SO_4	1G	1H	1I	1J	1K	1L
Saturated $CaSO_4$	21G	21H	21I	21J	21K	21L
	80% β-$2CaO.SiO_2$+20% of:					
	C_3A	C_5A_3	CA	C_3A_5	C_2F	C_4AF
0.15 *M* $MgSO_4$	2M	2N	2O	2P	2Q	2R
0.15 *M* Na_2SO_4	1M	1N	1O	1P	1Q	1R
Saturated $CaSO_4$	21M	21N	21O	21P	21Q	21R
	40% $3CaO.SiO_2$+40% β-$2CaO.SiO_2$+20% of:					
	C_3A	C_5A_3	CA	C_3A_5	C_2F	C_4AF
0.15 *M* $MgSO_4$	2T	2U	2V	2W	2X	2Y
0.15 *M* Na_2SO_4	1T	1U	1V	1W	1X	1Y
Saturated $CaSO_4$	21T	21U	21V	21W	21X	21Y

*C = CaO; S = SiO_2; A = Al_2O_3; F = Fe_2O_3

1. *Exposures to 0.15 M Solution of Magnesium Sulphate*

Figs. 4 and 5 give the expansion curves for the 1:10 mortars made from the various mixtures outlined in Table XI when exposed to a 0.15 *M* solution of magnesium sulphate. The expansion curves for the 1:10 silicate mortars are also given for comparison. Substituting 20% of the tricalcium silicate in the mortar with monocalcium aluminate (Curve 2I) or with $3CaO.5Al_2O_3$ (Curve 2J) causes a very rapid increase in the rate of expansion while the presence of 20% of tricalcium aluminate (Curve 2G) or 20% of $5CaO.3Al_2O_3$ (Curve 2H) also gives a very rapidly expanding unstable mortar. The rate of expansion for the first two mortars is actually higher than that for the corresponding mortars

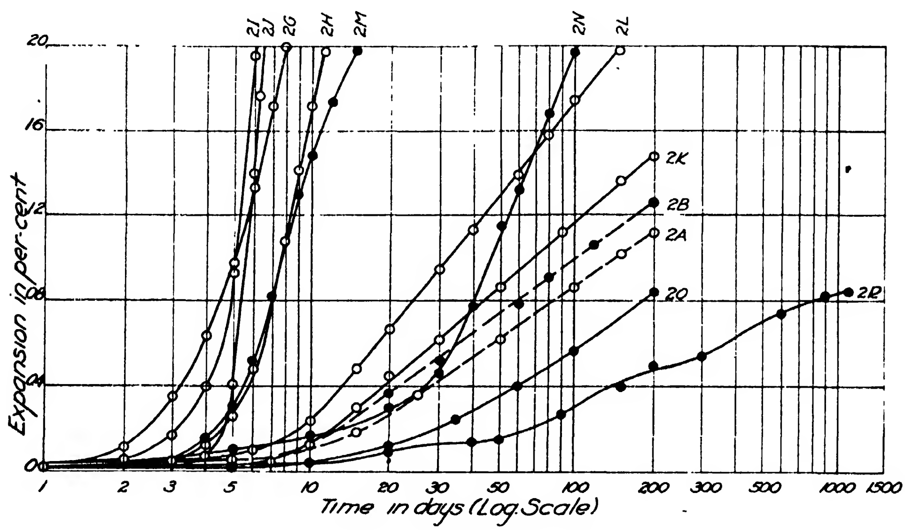

FIG. 4. *Expansion of 1:10 sand mortars made with mixtures of the calcium silicates, calcium aluminates, etc., as the cement, in 0.15 M* $MgSO_4$.

Legend: $A = 3CaO.SiO_2$; $B = \beta\text{-}2CaO.SiO_2$; $G = 80\%\ 3CaO.SiO_2 + 20\%\ 3CaO.Al_2O_3$. $H = 80\%\ 3CaO.SiO_2 + 20\%\ 5CaO.3Al_2O_3$; $I = 80\%\ 3CaO.SiO_2 + 20\%\ CaO.Al_2O_3$. $J = 80\%\ 3CaO.SiO_2 + 20\%\ 3CaO.5Al_2O_3$; $K = 80\%\ 3CaO.SiO_2 + 20\%\ 2CaO.Fe_2O_3$. $L = 80\%\ 3CaO.SiO_2 + 20\%\ 4CaO.Al_2O_3.Fe_2O_3$; $M = 80\%\ \beta\text{-}2CaO.SiO_2 + 20\%\ 3CaO.Al_2O_3$. $N = 80\%\ \beta\text{-}2CaO.SiO_2 + 20\%\ 5CaO.3Al_2O_3$; $O = 80\%\ \beta\text{-}2CaO.SiO_2 + 20\% CaO.Al_2O_3$ $R = 80\%\ \beta\text{-}2CaO.SiO_2 + 20\%\ 4CaO.Al_2O_3.Fe_2O_3$.

containing the aluminates alone and very much higher than that of the bars made from tricalcium silicate and sand (Curve 2A). The results obtained when the cement is composed of equal parts of tricalcium silicate and β-dicalcium silicate with 20% of the respective aluminates (Fig. 5, Curve 2T, 2U, 2V, 2W) are very similar except that below an expansion of 1% the rate of expansion increases with increasing percentage of lime in the aluminates. When the aluminates are added to β-dicalcium silicate only, the mix containing added tricalcium aluminate (Curve 2M) has a very high rate of expansion and the rate of expansion decreases rapidy with the decreasing percentage of lime in the aluminate present. Substitution of 20% of the β-dicalcium silicate with

$5CaO.3Al_2O_3$ affects the rate of expansion of the mortar only slightly at low expansions, but after reaching an expansion of 0.5% in 35 days the curve is almost as steep as that for tricalcium aluminate. On the other hand, there is a *decrease* in the rate of expansion when one compares a mortar of β-dicalcium silicate with one in which 20% of the β-dicalcium silicate is substituted by monocalcium aluminate, expansions of 0.5% and 0.85% requiring 28 and 70 days respectively, for the former, as against 78 and 300 days respectively, for the latter (Fig. 4, Curves 2B and 2O). A comparison with the cement containing 80% of β-dicalcium silicate and 20% of $3CaO.5Al_2O_3$ was not obtained as

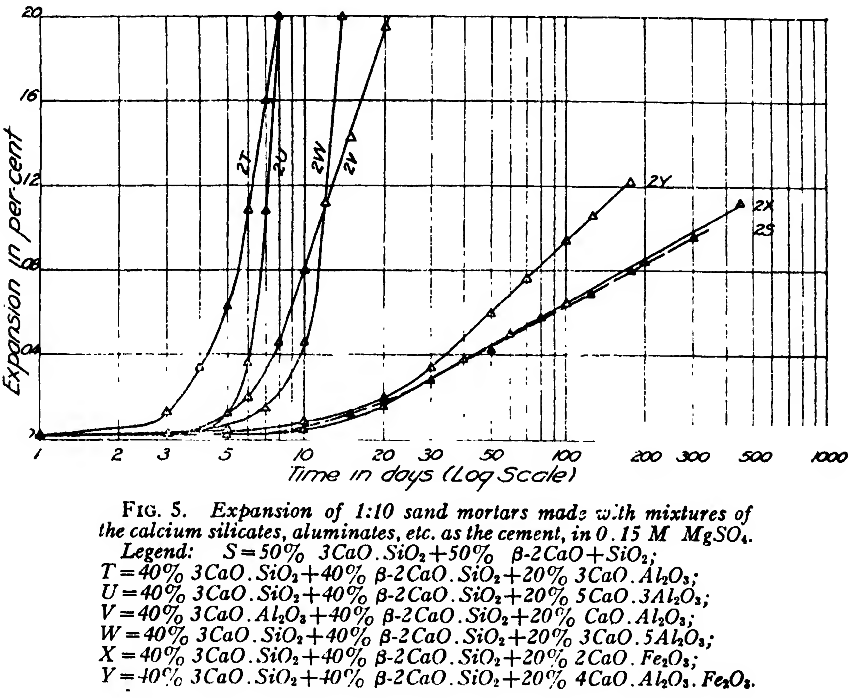

FIG. 5. *Expansion of 1:10 sand mortars made with mixtures of the calcium silicates, aluminates, etc. as the cement, in 0.15 M* $MgSO_4$.
Legend: $S = 50\%\ 3CaO.SiO_2 + 50\%\ \beta\text{-}2CaO + SiO_2$;
$T = 40\%\ 3CaO.SiO_2 + 40\%\ \beta\text{-}2CaO.SiO_2 + 20\%\ 3CaO.Al_2O_3$;
$U = 40\%\ 3CaO.SiO_2 + 40\%\ \beta\text{-}2CaO.SiO_2 + 20\%\ 5CaO.3Al_2O_3$;
$V = 40\%\ 3CaO.Al_2O_3 + 40\%\ \beta\text{-}2CaO.SiO_2 + 20\%\ CaO.Al_2O_3$;
$W = 40\%\ 3CaO.SiO_2 + 40\%\ \beta\text{-}2CaO.SiO_2 + 20\%\ 3CaO.5Al_2O_3$;
$X = 40\%\ 3CaO.SiO_2 + 40\%\ \beta\text{-}2CaO.SiO_2 + 20\%\ 2CaO.Fe_2O_3$;
$Y = 40\%\ 3CaO.SiO_2 + 40\%\ \beta\text{-}2CaO.SiO_2 + 20\%\ 4CaO.Al_2O_3.Fe_2O_3$.

the 1:10 bars were of such low strength when removed from the molds that all broke in handling. Pieces exposed to 0.15 *M* $MgSO_4$, however, seemed to increase in strength and after an exposure of seven months gave a tension of 87 lb. per sq. in., which is about the same as the tension for 1:10 bars of a high strength Portland cement stored in water.

Substitution of 20% of the tricalcium silicate by dicalcium ferrite or $4CaO.Al_2O_3.Fe_2O_3$ increases the rate of expansion of the mortar materially (Curves 2K and 2L), the effect of $4CaO.Al_2O_3.Fe_2O_3$ being greater than that of dicalcium ferrite. When 20% of the mixture of equal parts of tricalcium silicate and β-dicalcium silicate is substituted by $4CaO.Al_2O_3.Fe_2O_3$ there is only a slight increase in the rate of expansion of the mortar (Fig. 5, Curves 2Y and 2S) while a similar substitution by dicalcium ferrite does not produce an appreciable change. On the other hand, substitution of 20% of the β-dicalcium silicate in a 1:10 mortar by $4CaO.Al_2O_3.Fe_2O_3$ causes a very marked

decrease in the rate of expansion, the time required for a linear expansion of 0.5% being increased from 28 days to 7 months, (Fig. 4, Curves 2B and 2R), and the time for an expansion of 0.85% from 70 days to over 3 years (15 times). A comparison was not obtained with the cement containing 80% of β-dicalcium silicate and 20% of dicalcium ferrite on account of the low strength of the mortar specimens when removed from the molds. Pieces of 1:10 mortar bars exposed to 0.15 *M* magnesium sulphate seemed, however, to be unaffected after seven months' exposure.

Some fragmentary data are available as to the effect on the tensile strength of the mortar produced by the exposure of the bars to 0.15 *M* $MgSO_4$. These are given in Table XII. The figures in the column, "expansion", do not in some cases have much significance. The higher values in this column, such as those referring to mixtures containing aluminates, represent the expansion of the bars when they fell to pieces. The lower values represent the expansions at the time the determinations of tensile strength were made. No determinations of tension were made until after an exposure of 200 days so that no quantitative data are available for the rapidly expanding mortars containing mixtures of tricalcium silicate and the aluminates. From the notes made in each case when the measurements were discontinued it is possible to make an estimate of the relative tension. Those marked "very low" probably did not have a tension of above 5 lb. per sq. in., those marked "low" may have had at most twice that strength, and those marked "fairly firm" were slightly in excess of this.

TABLE XII*

EXPANSION AND TENSILE STRENGTH OF 1:10 MORTARS EXPOSED TO 0 15 *M* $MgSO_4$

Serial No.	Cement	Expansion, %	Time of exposure, days	Tensile strength, lb. per sq. in
2A	C_3S	1 12	200	65
2B	β-C_2S	1 26	200	38
2S	50% C_3S+50% β-C_2S	1 05	325	42
2G	80% C_3S+20% C_3A	4 86	26	Low
2H	80% C_3S+20% C_5A_3	4 69	26	Low
2I	80% C_3S+20% CA	6 27	10	Very low
2J	80% C_3S+20% C_3A_5	5 50	10	Very low
2K	80% C_3S+20% C_2F	1 48	200	48
2L	80% C_3S+20% C_4AF	2 04	160	Bars firm
2T	40% C_3S+40% β-C_2S+20% C_3A	3 08	11	Low
2U	40% C_3S+40% β-C_2S+20% C_5A_3	3 58	10	Very low
2V	40% C_3S+40% β-C_2S+20% CA	3 00	45	Bars fairly firm
2W	40% C_3S+40% β-C_2S+20% C_3A_5	3 32	16	Low
2X	40% C_3S+40% β-C_2S+20% C_2F	1 11	450	86
2Y	40% C_3S+40% β-C_2S+20% C_4AF	>1 22	200	60
2M	80% β-C_2S+20% C_3A	3 05	35	Bars fairly firm
2N	80% β-C_2S+20% C_5A_3	>2 10	210	60
2O	80% β-C_2S+20% CA	>0 85	300	81
2P	80% β-C_2S+20% C_3A_5		200	87
2Q	80% β-C_2S+20% C_2F		—	No test
2R	80% β-C_2S+20% C_4AF	0 85	1100	No test

*$C = CaO$; $S = SiO_2$; $A = Al_2O_3$; $F = Fe_2O_3$.

It will be seen from Table XII that all the mortars which expanded rapidly also lost their strength rapidly, while those which expanded slowly retained considerable strength even after long exposures to the sulphate solution. Further, the substitution of 20% of the mixed silicates by either dicalcium ferrite or $4CaO.Al_2O_3.Fe_2O_3$ or the substitution of 20% of β-dicalcium silicate by either monocalcium aluminate or $3CaO.5Al_2O_3$ gave mortars which retained their strength better than those made with the corresponding silicates alone, and in some cases after an exposure of from 6 to 15 months gave a tension nearly equal to that of Portland cement of the same mix stored in fresh water.

2. *Exposures to Saturated $CaSO_4$ and to 0.15 M Na_2SO_4*

Figs. 6 and 7 give the expansion data for this series of experiments. When 1:10 mortars made from cements containing 80% of the calcium silicates with

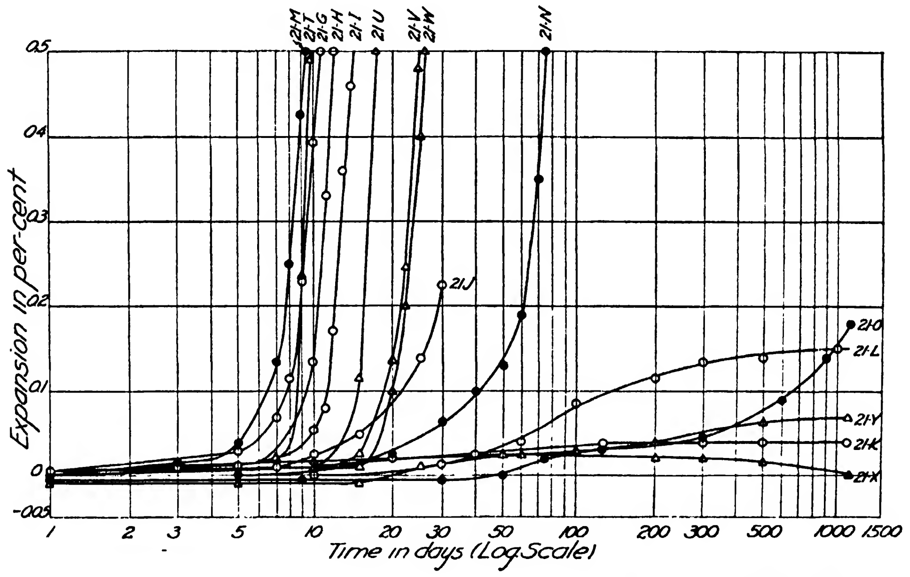

FIG. 6 *Expansion of 1:10 sand mortars made with mixtures of the calcium silicates, aluminates, etc. as the cement, in a saturated solution of $CaSO_4$.*
Legend: G, H, I, J, K, and L = 80% $3CaO.SiO_2$ with 20% of $3CaO.Al_2O_3$, $5CaO.3Al_2O_3$, $CaO.Al_2O_3$, $3CaO.5Al_2O_3$, $2CaO.Fe_2O_3$ and $4CaO.Al_2O_3.Fe_2O_3$, respectively. M, N, and O = 80% β-$2CaO.SiO_2$ with 20% of $3CaO.Al_2O_3$, $5CaO.3Al_2O_3$ and $CaO\ Al_2O_3$, respectively. T, U, V, W, X and Y = 40% $3CaO.SiO_2$ + 40% β-$2CaO.SiO_2$ with 20% of $3CaO.Al_2O_3$, $5CaO.3Al_2O_3$, $CaO.Al_2O_3$, $3CaO.5Al_2O_3$, $2CaO.Fe_2O_3$ and $4CaO.Al_2O_3.Fe_2O_3$, respectively.

20% of the various calcium aluminates were exposed to a saturated solution of calcium sulphate the rate of expansion was in general low for the first few days followed by a period of more rapid expansion. Considering each of the three basic silicate cements, (1) 100% C_3S, (2) 50% C_3S + 50% β-C_2S, (3) 100% β-C_2S, in turn, one finds that the length of the period of slow expansion was increased as the proportion of lime in the substituting aluminate decreased, *i.e.*, in the order $3CaO.Al_2O_3 < 5CaO.3Al_2O_3 < CaO.Al_2O_3 < 3CaO.5Al_2O_3$. (Curves G, H, I, and J; T, U, V, and W; M, N, and O). In the case of the

mortars highest in tricalcium silicate there was a marked amount of crumbling and an early decrease in strength when exposed to 0.15 M Na_2SO_4 as shown by the fact that the expansion curves come to an end at very low expansions (Curves 1G, 1H, 1I and 1J). The mortar containing 80% of tricalcium silicate and 20% of monocalcium aluminate actually crumbled to pieces after an exposure of six days after a linear expansion of slightly over 0.03% (1I).

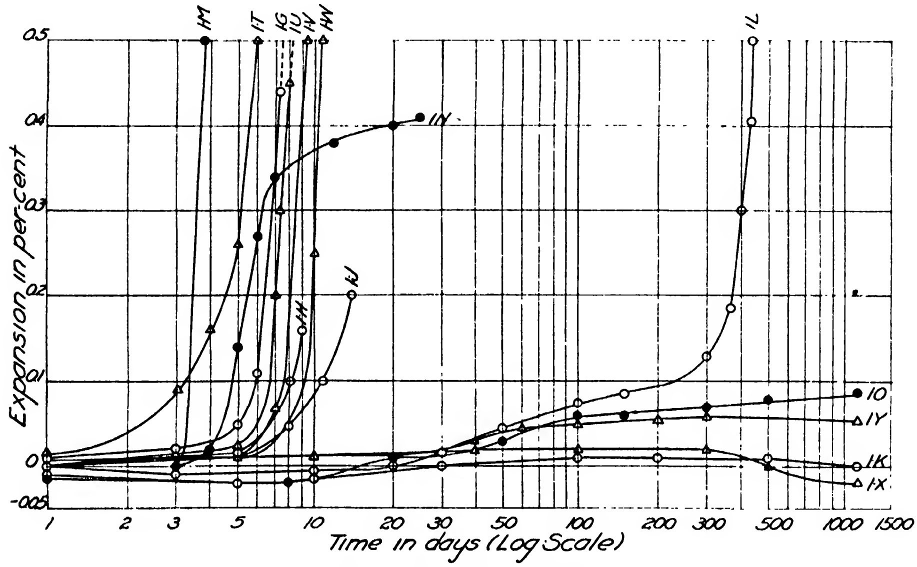

FIG. 7. *Expansion of 1:10 sand mortars made with mixtures of the calcium silicates, aluminates, etc. as the cement, in a 0.15 M Na_2SO_4.*
Legend: G, H, J, K and L = 80% $3CaO.SiO_2$ with 20% of $3CaO.Al_2O_3$, $5CaO.3Al_2O_3$, $3CaO.5Al_2O_3$, $2CaO.Fe_2O_3$ and $4CaO.Al_2O_3.Fe_2O_3$, respectively. M, N and O = 80% β-$2CaO.SiO_2$ with 20% $3CaO.Al_2O_3$, $5CaO.3Al_2O_3$ and $CaO.Al_2O_3$, respectively. T, U, V, W, X and Y = 40% $3CaO.SiO_2$ + 40% β-$2CaO.SiO_2$ with 20% of $3CaO.Al_2O_3$, $5CaO.3Al_2O_3$, $CaO.Al_2O_3$, $3CaO.5Al_2O_3$, $2CaO.Fe_2O_3$ and $4CaO.Al_2O_3.Fe_2O_3$, respectively.

It is rather remarkable that while 1:10 sand mortars made with tricalcium silicate and similar mortars made with tricalcium silicate and β-dicalcium silicate in equal proportions were apparently not affected by exposures to a saturated solution of calcium sulphate or a 0.15 M solution of sodium sulphate for a period of three years, the corresponding mortars in which only 20% of the silicate was substituted by the various calcium aluminates expanded in these solutions as a rule faster than similar mortars made from the corresponding calcium aluminate. The same applies to mortars made with a cement containing 80% of the mixed silicates, the balance being composed of the mixed aluminates.

The substitution of 20% of the tricalcium silicate by dicalcium ferrite and a similar substitution in the case of the cement containing equal proportions of tricalcium silicate and β-dicalcium silicate gave mortars which showed almost no change in expansion as compared with the corresponding silicate mortars at the end of three years exposure (Curves 1K, 1X, 21K, 21X). Similar substitutions

by the compound $4CaO.Al_2O_3.Fe_2O_3$ increased the rate of expansion of the tricalcium silicate mortar materially, giving a linear expansion of 0.7% in 16 months when exposed to 0.15 M Na_2SO_4 (Curve 1L) and an expansion of 0.16% in three years in the saturated solution of calcium sulphate (Curve 21L). On the other hand, in the case of the mortar containing tricalcium silicate and β-dicalcium silicate in equal proportions, the substitution by $4CaO.Al_2O_3.Fe_2O_3$ increased the expansion only very slightly as compared with the mortar made from the pure silicates (namely, 0.05% in 0.15 M Na_2SO_4, Curve 1Y, and 0.09% in saturated $CaSO_4$, Curve 21Y, at the end of three years as compared with 0.00% and 0.02% for the pure silicate mortar in the respective solutions, Curves 1S and 21S).

The 1:10 mortar bars made from the cement containing 80% of β-dicalcium silicate with 20% of the various aluminates gave rates of expansion which were in the same order as those for the corresponding tricalcium silicate plus aluminate mortars. The rate of expansion for the bars containing tricalcium aluminate was very rapid in both solutions (Curves 1M and 21M). The expansion of the mortar containing 20% of $5CaO.3Al_2O_3$ (Curves 1N and 21N) was very much slower, and the bars were fairly resistant to the action of the solutions, as shown by the fact that the tension of the bars after exposure for seven months was 67 lb. per sq. in. for those in 0.15 M Na_2SO_4 and 71 lb. per sq. in. for those in saturated $CaSO_4$. The mortar made from the cement of the composition 80% β-$2CaO.SiO_2$ + 20% $CaO.Al_2O_3$ was extremely resistant to both solutions. When exposed to saturated $CaSO_4$ it reached a linear expansion of 0.1% in 24 months (Curve 21O), while in 0.15 M Na_2SO_4 it had not reached this expansion after three years' exposure (Curve 1O). Bars containing 80% β-$2CaO.SiO_2$ + 20% $3CaO.5Al_2O_3$ were not measured but appeared to be in good condition after seven months' exposure and had then a tension of 43 lb. per sq. in. for those in 0.15 M Na_2SO_4 and 60 lb. for those in the saturated solution of $CaSO_4$.

No measurements of expansion of the bars containing dicalcium ferrite or $4CaO.Al_2O_3.Fe_2O_3$ added to β-dicalcium silicate were obtained. Examination of pieces of bars exposed to the solutions indicated, however, that they were highly resistant to the action of the solutions. The bars made with 80% of β-dicalcium silicate and 20% of $4CaO.Al_2O_3.Fe_2O_3$ had a tension of 48 lb. per sq. in. after seven months' exposure to 0.15 M Na_2SO_4. In considering the tension data one must bear in mind that the addition of dicalcium ferrite and $4CaO.Al_2O_3.Fe_2O_3$ to the silicates gave bars which had very low strengths when placed in the solutions. The tensions obtained after the above exposures of these bars were probably in all cases higher than at the time of immersion in the solutions.

Discussion of the Results Obtained with Mortars of Mixed Silicates and Aluminates

The most interesting observation made is the striking difference between the behavior on exposure to sulphate solutions of mortars made from mixtures of the aluminates with tricalcium silicate as the basic material on the one hand

and with β-dicalcium silicate as the basic material on the other The expansion obtained with mortars made of such mixtures cannot be calculated from the behavior of the corresponding mortars made from the pure substances and exposed to the same solutions under similar conditions. The curves already given show that when the cementing material contained 80% of tricalcium silicate, the balance being in turn tricalcium aluminate, $5CaO.3Al_2O_3$, monocalcium aluminate and $3CaO.5Al_2O_3$, the resulting mortars were extremely unstable in sulphate solutions, exhibiting very rapid volume changes and loss of strength out of proportion to what would have been expected from the behavior of similar mortars made from the individual substances. This was also the case when the tricalcium silicate was substituted by a mixture of tricalcium silicate and β-dicalcium silicate in equal proportions, with the aluminate remaining the same. In general it may be said that the presence of any of the calcium aluminates in a cement containing a large quantity of tricalcium silicate renders mortars made from it very unstable in sulphate solutions. It should be noted here that aluminates low in lime are not likely to be present in the product of a mix high in lime calcined until equilibrium is attained. Incomplete burning or the firing of a kiln at a temperature above the decomposition point of tricalcium aluminate might cause the presence of $5CaO.3Al_2O_3$ in the calcined product.

The picture is changed very materially when one considers the presence of the aluminates in a cement of which β-dicalcium silicate is the base. The mortar containing a considerable quantity of tricalcium aluminate was still quite unstable in all the sulphate solutions. The mortar made with a cement containing 80% β-$2CaO.SiO_2$ + 20% $5CaO.3Al_2O_3$ showed marked signs of increased stability. The expansion was slowed down, although still high, and the mortar retained its strength remarkably well. Tension tests on the 1:10 mortars gave after seven months' exposure 60 lb. per sq.in. in 0.15 M $MgSO_4$, 67 lb. per sq. in. in 0.15 M Na_2SO_4 and 71 lb. per sq. in. in saturated $CaSO_4$. Admixture of monocalcium aluminate, which in the presence of large amounts of tricalcium silicate gave perhaps the most unstable mortar, gave a remarkable result when β-dicalcium silicate was the basic material. The 1:10 mortar made with the cement containing 80% of β-dicalcium silicate and 20% of monocalcium aluminate had a much higher stability in 0.15 M $MgSO_4$ than the corresponding mortar made with the pure silicate. At the end of seven months' exposure, it gave an expansion of 0.85% and after 10 months' exposure, a tension of 80 lb. per sq. in. as against an expansion of 1.26% and a tension of 38 lb. per sq. in. for the pure silicate mortar at the end of seven months' exposure. The expansion of this mortar in 0.15 M Na_2SO_4 and saturated $CaSO_4$ was very low indeed (0.09% and 0.18% respectively at the end of three years' exposure) although somewhat greater than that of the pure silicate mortar in the same solutions.

The anomalous behavior of mixtures containing tricalcium silicate and β-dicalcium silicate respectively was also evident when $4CaO.Al_2O_3,Fe_2O_3$ was present in the cement mixture. Sand mortar (1:10) made with a cement composed of 80% of tricalcium silicate and 20% of $4CaO.Al_2O_3,Fe_2O_3$ expanded

in all three sulphate solutions much faster than the corresponding mortar made with a cement of pure tricalcium silicate. This was especially marked in 0.15 *M* $MgSO_4$ and after long exposure to 0.15 *M* Na_2SO_4. On the other hand, when β-dicalcium silicate was substituted for the tricalcium silicate, the amount of $4CaO.Al_2O_3,Fe_2O_3$ remaining the same, the mortar expanded much more slowly in 0.15 *M* $MgSO_4$ than the corresponding sand mortar made with pure β-dicalcium silicate (0.84% in three years as compared with 0.84% in 70 days). The expansion curves for this mortar in 0.15 *M* Na_2SO_4 and saturated $CaSO_4$ are not available.

It is evident that the compound $4CaO.Al_2O_3.Fe_2O_3$ is not as sensitive to the adverse effect of the presence of tricalcium silicate in the mortar as are the calcium aluminates. When $4CaO.Al_2O_3.Fe_2O_3$ was present to the extent of 20% in the cement containing tricalcium silicate and β-dicalcium silicate in equal proportions, the speeding up of the expansion, as compared with that of the pure silicate cement, was marked only in the 0.15 *M* $MgSO_4$. The expansions at the end of three years' exposure to 0.15 *M* Na_2SO_4 and saturated $CaSO_4$ were 0.05% and 0.07%, respectively, which were only slighty in excess of the maximum expansion of the mortar made with the silicate. The figures in Table XIII illustrate the effect of the silicate on the expansion in 0.15 *M* $MgSO_4$ of mortars containing $4CaO.Al_2O_3.Fe_2O_3$.

When dicalcium ferrite was substituted for the $4CaO.Al_2O_3.Fe_2O_3$ in the above mortars there was an increase in the rate of expansion as compared with that of the corresponding silicate mortar only in the case of the mortar made from the cement containing 80% of tricalcium silicate and only for exposure to 0.15 *M* $MgSO_4$. The expansion curve for the mortar containing the silicate mixture with the dicalcium ferrite was practically the same as for the corresponding silicate mortar. The substitution of 20% of the silicates by dicalcium ferrite had no appreciable effect on the expansion in 0.15 *M* Na_2SO_4 or saturated $CaSO_4$.

TABLE XIII

EFFECT OF TRICALCIUM SILICATE ON THE EXPANSION OF MORTARS CONTAINING $4CaO.Al_2O_3.Fe_2O_3$, AND IMMERSED IN 0.15 *M* $MgSO_4$

Cement in 1:10 sand mortar	C_3S	80% C_3S 20% C_4AF	50% C_3S 50% β-C_2S	40% C_3S 40% β-C_2S 20% C_4AF	β-C_2S	80% β-C_2S 20% C_4AF
Time in days necessary for expansion of 0.84%	98	56	195	85	70	1095 (3 yr.)

The adverse effect produced by a high content of tricalcium silicate is thus most evident when the mortar is exposed to a solution of magnesium sulphate. The effect is, however, probably just as great in solutions of sodium and calcium sulphate but becomes noticeable earlier in solutions of magnesium sulphate on account of the aggressive action of this salt on the silicates. In each of the solutions the effect is greatest with cements containing the calcium aluminates,

much smaller when the compound $4CaO.Al_2O_3.Fe_2O_3$ is present, and where dicalcium ferrite is present, is apparent only in the cement containing 80% of tricalcium silicate exposed to 0.15 M $MgSO_4$. On the other hand, the beneficial effect on the resistance to sulphates observed when β-dicalcium silicate is the basic substance, is apparent in 0.15 M $MgSO_4$ when 20% of monocalcium aluminate is present, appears to be more marked with the aluminate, $3CaO.5Al_2O_3$, and is very pronounced with the compound $4CaO.Al_2O_3.Fe_2O_3$.

Application to Commercial Portland Cement

Fig. 8 gives the expansion curves for the synthetic cements which showed, in the form of a 1:10 sand mortar, a very materially increased resistance to the action of sulphates as compared with commercial Portland cements. Since

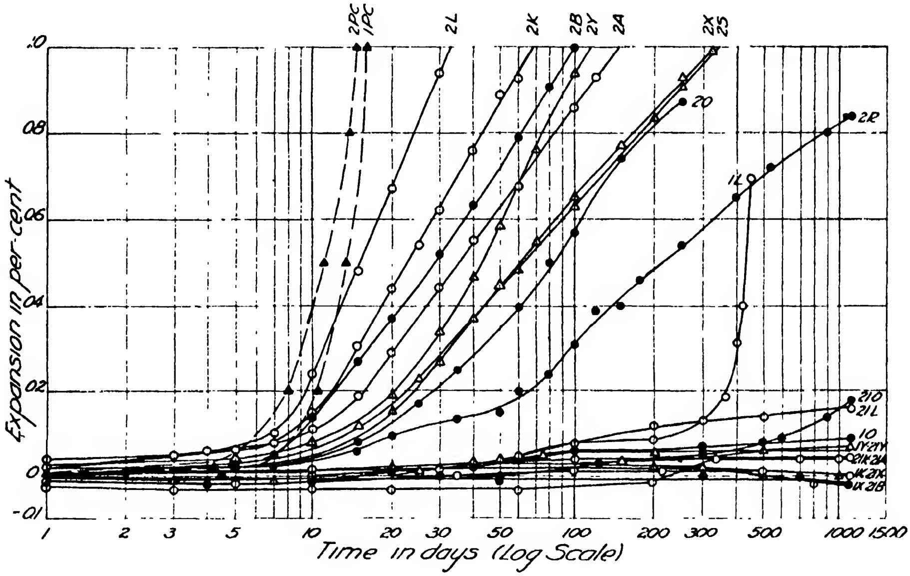

FIG. 8. *Expansion in sulphate solutions of 1:10 sand mortars made with mixtures of calcium silicates, aluminates, etc. which are more resistant to the solutions than the average commercial Portland cement.*

Legend: P.C. = *Portland cement (average of eight cements).* $A = 3CaO.SiO_2$; $B = \beta\text{-}2CaO.SiO_2$; *K and L* = 80% $3CaO.SiO_2$ *with* 20% *of* $2CaO.Fe_2O_3$ *and* $4CaO.Al_2O_3.Fe_2O_3$, *respectively; O and R* = 80% $\beta\text{-}2CaO.SiO_2$ *with* 20% *of* $CaO.Al_2O_3$ *and* $4CaO.Al_2O_3.Fe_2O_3$, *respectively.* S = 50% $3CaO.SiO_2$ + 50% $\beta\text{-}2CaO.SiO_2$. *X and Y* = 40% $3CaO.SiO_2$ + 40% $\beta\text{-}2CaO.SiO_2$ *with* 20% *of* $2CaO.Fe_2O_3$ *and* $4CaO.Al_2O_3.Fe_2O_3$, *respectively.* 1 = 0.15 M Na_2SO_4; 2 = 0.15 M $MgSO_4$; 21 = *Saturated* $CaSO_4$.

their resistance was lower or only slightly in excess of that of Portland cement, the following synthetic cements are omitted: All those containing 20% of the calcium aluminates with 80% of tricalcium silicate or with the mixture of 40% of tricalcium silicate and 40% of β-dicalcium silicate, and those containing either 20% of tricalcium aluminate or 20% of $5CaO.3Al_2O_3$ with 80% of β-dicalcium silicate. The resistance of the last named is, however, higher than that of Portland cement. A few of the synthetic cements which gave evidence of great resistance to sulphate solutions but which gave such a weak 1:10 sand

mortar that all the bars were damaged when they were removed from the molds also had to be omitted. These include the mixture of 80% of β-dicalcium silicate and 20% of $3CaO.5Al_2O_3$, 80% of β-dicalcium silicate and 20% of dicalcium ferrite. There is, for the same reason, only a partial set of data for the mixture containing 80% of β-dicalcium silicate and 20% of $4CaO.Al_2O_3.Fe_2O_3$. A few curves very close to the horizontal axis are omitted owing to crowding. These include curves for the mortar made with tricalcium silicate and exposed to 0.15 *M* Na_2SO_4, the β-dicalcium silicate mortar exposed to 0.15 *M* Na_2SO_4 and the mortar made with the mixture of the two silicates and exposed to 0.15 *M* Na_2SO_4 and saturated $CaSO_4$. Included also are curves for 1:10 Portland cement mortar exposed to 0.15 *M* Na_2SO_4 (Curve 1 PC) and 0.15 *M* $MgSO_4$ (Curve 2 PC). These represent the average of the data obtained with eight commercial Portland cements manufactured in eight different cement plants in Canada and the United States.

The gypsum present in commercial Portland cements is of importance in regulating the set of the cement. This action appears to be due at least in part to modification of the solubility and hydration of the calcium aluminate in the cement (5). Test pieces made with a ground Portland cement clinker without the addition of gypsum generally give lower strengths than similar test pieces made with the same cement after addition of gypsum. No gypsum was added to any of the experimental cements described in this paper. It may be concluded, therefore, that in the case of at least some of the composite mixes, especially those containing mixtures of the silicates and calcium aluminates, the strength of the test pieces was lower than would have been the case if gypsum had been added. The effect of this on the comparative results obtained is, however, probably very slight, as the strength of the test pieces—once they are strong enough to be handled without breakage—is of minor importance under the conditions of the experiments. When concrete test pieces are exposed to sulphate solutions under conditions of alternate freezing and thawing, or where the test pieces are partly exposed to the air, the porosity and strength are of very great importance in determining the permanency. When, however, the test pieces are completely immersed in the sulphate solution at constant temperature the strength plays only a very secondary role. Many examples showing this are available. The 1:10 mortar made with γ-dicalcium silicate, which had a tensile strength of only 10 lb. per sq. in. after three years' curing in water, stood up extremely well in the three sulphate solutions, giving a slower rate of expansion in solutions of calcium and magnesium sulphate than similar mortar made with tricalcium silicate, although the latter had many times the strength of the former. The mortar bars made with cement I (80% $3CaO.SiO_2$ + 20% $CaO.Al_2O_3$) which had a tensile strength of 60 lb. per sq. in. when exposed to 0.15 *M* $MgSO_4$ expanded 1% in five days, while those made with cement K (80% $3CaO.SiO_2$ + 20% $2CaO.Fe_2O_3$) which had a tensile strength of 47 lb. per sq. in. when exposed, required 70 days to expand the same amount. The bars made with cement R (80% β-$2CaO.SiO_2$ + 20% $4CaO.Al_2O_3.Fe_2O_3$), having a tensile strength of 20 lb. per sq. in., expanded only 0.84% after an exposure to the same solution for three years. It thus

appears that the differences in the chemical reactions have the predominating influence and the strength of the specimens only a minor one. Experiments with a large number of commercial Portland cements have shown also that the strength developed by the cement has only a very slight influence on the rate of expansion or the loss of strength of its test pieces, when exposed to sulphate solutions under conditions of complete immersion at a constant temperature. It may be assumed, therefore, that while the presence of gypsum would have modified somewhat the strength of the mortars made from the composite cements, nevertheless the effect on the expansion would have been slight, and that in general the information obtained as to the resistance of the various compounds to sulphate solutions is applicable to commercial cements.

In considering the significance of the actual time required for any particular linear expansion of the experimental mortars it must be remembered that the very lean mortar and the conditions of treatment and exposure render the tests extremely severe. A comparison of the expansion curves for the experimental mortars with those for similar mortars made of commercial Portland cements makes it possible to obtain a quantitative measure or ratio of the relative resistance of any of the composite cements and Portland cement. Since this comparison is based only on the linear expansion of the mortars and since it was found that the tensile strength of these fell off much more slowly with increasing length than that of similar mortars made with Portland cement, it is evident that the ratio obtained gives a minimum value for increased resistance.

TABLE XIV

EXPANSION OF MORTAR BARS IN 0.15 *M* Na_2SO_4

Cement	Ratio, Cement : sand			
	1:10	1:5	1:3	1:2
	Time in days for expansion of 0.50%			
A	9.2	12.3	31.8	125
B	10.0	19.2	89	450
Ratio B/A	1.09	1.56	2.80	3.60

When considering the relation between the life of a specimen of a 1:10 sand mortar exposed to sulphate action and that of concrete or rich mix there are two facts which must be remembered: First, when the proportion of cement in a mortar is increased, the resistance of the mortar increases up to a certain limit; second, a slight difference in the relative resistance of two cements, as shown by expansion measurements of very lean mortars, may be greatly magnified when the richness of mix is increased. This is illustrated by the data obtained with two commercial Portland cements A and B, as given in Table XIV (19).

Assuming the resistance to be directly proportional to the time required for an expansion of 0.50%, one finds that as the mix changes from 1:10 to 1:2 the

resistance of the less resistant cement, A, increases 13 times while the increase for the more resistant cement, B, is 45 times. Considering the 1:10 mortar, it appears that Cement B is 9% more resistant than Cement A while from the results with the 1:2 mortar Cement B appears to be 360% more resistant than Cement A. When one exposes the mortar to 0.15 *M* $MgSO_4$ instead of to Na_2SO_4 the decrease in the rate of expansion with increasing richness of mix is even more marked.

Table XV gives the time required for an expansion of 0.50% for the mortars made from the experimental cements (Fig. 8) which showed a higher resistance than the average of the eight commercial Portland cements when exposed to 0.15 *M* $MgSO_4$. It also gives the ratio for each of the mortars on the basis of unity for the average Portland cement. The cements are also arranged in order of increasing resistance to 0.15 *M* $MgSO_4$. It will be seen that the time for an expansion of 0.5% increases from 140% in the case of Cement L (80% $3CaO.SiO_2$ + 20% $4CaO.Al_2O_3.Fe_2O_3$) to 1910% in the case of Cement R (80% β-$2CaO.SiO_2$ + 20% $4CaO.Al_2O_3.Fe_2O_3$) as compared with 100% for the average of the eight commercial Portland cements.

The available data on the tensile strength of the mortar bars after exposures for various time intervals to 0.15 *M* $MgSO_4$ are also given in Table XV. Although these were not made systematically at a definite linear expansion, they give valuable information when considered with reference both to the corresponding linear expansion and length of exposure to the solution. The retention of strength by the mortars made from the synthetic cements, after long periods of exposure and at high expansion, is very striking when compared with the average for the Portland cements.

TABLE XV

DATA ON EXPANSION AND TENSILE STRENGTH OF 1:10 MORTARS IN 0.15 *M* $MgSO_4$

Data	Cements									
	P.C.	L	K	B	A	Y	X	S	O	R
Time in days for expansion of 0.50%	11	15.5	23.0	29	35	43	60	65	85	210
Ratio (time for P.C. = 1)	1.0	1.4	2.1	2.6	3.2	3.9	5.5	5.9	7.7	19.1
Expansion when broken, %	1.0	2.04	1.48	1.26	>1.12	1.22	1.11	1.05	0.85	0.85
Time of exposure in days	15	160	200	200	200	200	450	325	300	1100
Tensile strength, lb. per sq. in.	10	*	48	38	65	60	86	42	81	†
Blank, 3 yr. in water, tensile strength lb. per sq. in.		>45	>47	–	–	102	92	73	–	–

*Not determined, bars firm.
†Not determined, bars still being measured.

Reference to Figs. 4, 5, 6, and 7 shows that for the synthetic cements of low resistance to sulphates the rate of expansion of the 1:10 mortars in 0.15 *M* $MgSO_4$ does not differ much from that in 0.15 *M* Na_2SO_4. The same applies to 1:10 mortars made with Portland cement (Fig. 8, Curves 2 P.C. and 1 P.C.).

On the other hand it is evident (Fig. 8) that the more resistant cements have developed an extremely high resistance to the action of solutions of sodium sulphate and calcium sulphate as compared with their resistance to 0.15 *M* $MgSO_4$. In only one case (1L, 80% $3CaO.SiO_2$ + 20% $4CaO.Al_2O_3.Fe_2O_3$ in 0.15 *M* Na_2SO_4) has one of the experimental mortars of Fig. 8 reached an expansion of 0.50% by the end of the three-year period of exposure, the time required being 32 times that of the average for the Portland cements. The mortar having the next greatest expansion (21O, 80% β-$2CaO.SiO_2$ + 20% $CaO.Al_2O_3$ in saturated $CaSO_4$) has required 110 times as long as the Portland cements for a linear expansion of 0.09%.

In view of the change in the resistance of Portland cement mortars when the richness of mix is increased, and the results obtained with 1:5 sand mortars of the pure silicates, one might interpret the data of Fig. 8 and Table XV, with reference to concretes of rich mix made with these cements, as follows:

Cements L and K would show very much increased resistance to the action of sulphate solutions as compared with concrete made from the usual commercial Portland cements. Cement L (80% $3CaO.SiO_2$ + 20% $4CaO.Al_2O_3.Fe_2O_3$) would, however, be subject to failure in water containing magnesium sulphate and probably after very long exposure to water containing sodium sulphate while some deterioration might take place in saturated calcium sulphate. Cement K (80% $3CaO.SiO_2$ + 20% $2CaO.Fe_2O_3$) while more resistant to solutions of magnesium sulphate would yet be subject to failure, but would give a concrete which is practically permanent in solutions of sodium and calcium sulphates.

Cements X and Y, while subject to the action of magnesium sulphate, would, in the form of rich concrete, have a life many times that of ordinary Portland cement, and under moderately severe conditions of exposure would probably approach permanency, especially in the case of rich concrete made with Cement X (40% $3CaO.SiO_2$ + 40% β-$2CaO.SiO_2$ + 20% $2CaO.Fe_2O_3$). Cement Y (40% $3CaO.SiO_2$ + 40% β-$2CaO.SiO_2$ + 20% $4CaO.Al_2O_3.Fe_2O_3$) would be somewhat more vulnerable. Both cements would give a concrete which would be practically permanent under exposure to water containing sodium or calcium sulphate.

Cement O (80% β-$2CaO.SiO_2$ + 20% $CaO.Al_2O_3$) would be slightly more resistant to solutions of magnesium sulphate than Cement X. It would, however, be less resistant than either X or Y to the action of calcium sulphate.

Cement R (80% β-$2CaO.SiO_2$ + 20% $4CaO.Al_2O_3.Fe_2O_3$) although slightly attacked in the form of a lean mortar by solutions of magnesium sulphate would have such a long life as to be practically immune in the form of a rich concrete. The early strength would, however, be very low.

It is now generally accepted that the compounds present is a well-burned Portland cement clinker are tricalcium silicate ($3CaO.SiO_2$), β-dicalcium silicate (β-$2CaO.SiO_2$), the compound $4CaO.Al_2O_3.Fe_2O_3$ and tricalcium aluminate ($3CaO.Al_2O_3$) while the magnesium oxide is probably present in the free state (6, 7, 11, 12). The fluxing materials are the iron compound and the tricalcium aluminate. The presence of magnesium oxide also lowers the

temperature of clinker formation (6). If there is more than sufficient iron oxide present to combine with all the alumina to form $4CaO.Al_2O_3.Fe_2O_3$, the excess of iron forms dicalcium ferrite ($2CaO.Fe_2O_3$). This is the case in iron-ore cement which was developed by Michaelis. The modern tendency in the manufacture of Portland cement is to increase the percentage of lime in the raw mix so as to increase the proportion of tricalcium silicate in the product and thus obtain high early strength. The same applies to iron-ore cements which tend to have a very low early strength unless high in tricalcium silicate and finely ground.

It is evident from Fig. 8 and Table XV that one cannot expect to obtain a cement of the Portland cement type high in tricalcium silicate which is at the same time very highly resistant to solutions of either magnesium or sodium sulphate. If the necessary fluxing material in the raw mix is alumina, with little iron present, the resulting cement high in tricalcium silicate will be extremely unstable in all three sulphate solutions. If the amount of alumina is reduced and more iron is present, to give fluxing material favoring combination of the silica and lime, the resistance to all the sulphate solutions is increased, but the limiting value obtained when there is enough iron present to combine with all the alumina, falls far short of the resistance of the basic tricalcium silicate. If the alumina is eliminated from the raw mix and the fluxing material is dicalcium ferrite, the resulting iron-ore cement still falls short of the resistance of pure tricalcium silicate when exposed to solutions of magnesium sulphate, but is very resistant to the action of solutions of sodium and calcium sulphate.

It is thus clearly indicated that the first requirement in order to produce a highly resistant cement is to reduce the amount of tricalcium silicate it contains. If this is reduced so that there are equal proportions of the two basic silicates and the fluxing material is alumina the cement will still be extremely unstable in sulphate solutions. If the alumina is now reduced in amount and the iron is increased until all the alumina has combined with the ferric oxide, a cement which is probably more resistant to magnesium sulphate than pure tricalcium silicate is produced (Curves 2Y and 2A). The expansion is, however, greater than for the cement of the mixed silicates (2S). The effect of solutions of sodium and calcium sulphate on this cement is quite small (Curves 1Y and 21Y). The corresponding iron-ore cement produced by eliminating the alumina from the mix and substituting ferric oxide as flux gives a cement which is as stable in $0.15M$ $MgSO_4$ as the basic silicate mixture (Curves 2X and 2S) and practically immune to the action of sodium and calcium sulphate (Curves 1X and 21X).

In order to obtain a cement which is more highly resistant to magnesium sulphate than the basic silicates, it is necessary to reduce the proportion of tricalcium silicate to less than one-half of the amount of silicates present in the cement. How far the resistance can be safely increased in this way depends on the requirements of early strength. Apart from requirements of strength one would expect that the most resistant material would be obtained in the case of Portland cement when enough iron oxide is present to combine with

all the alumina (Curve 2R) and in the case of iron-ore cement, when all the alumina is displaced by iron oxide, all the silica, in both cases, being present as dicalcium silicate.

It has already been shown that a mortar made from a cement containing tricalcium silicate with any of the calcium aluminates present is very unstable in sulphate solutions. It follows that high alumina cements should contain no tricalcium silicate and that the silica should be present in the form of β-dicalcium silicate. Further, mortar containing no tricalcium silicate but containing dicalcium silicate with tricalcium aluminate or $5CaO.3Al_2O_3$ is also unstable in sulphate solutions. Thus, high alumina cement, in order to be resistant to sulphates, should contain neither of these aluminates. This narrows the ideal composition of this type of cement to the extent that the silica should be present as β-dicalcium silicate and the alumina as either monocalcium aluminate or $3CaO.5Al_2O_3$ or both. The lower early strength obtained with mixtures of β-dicalcium silicate and $3CaO.5Al_2O_3$ suggests that monocalcium aluminate is preferable as the main constituent of high alumina cement. The tendency of lean sand mortars of $3CaO.5Al_2O_3$ to disintegrate on lengthy exposures to fresh water, which has been repeatedly observed in this laboratory, further confirms this view.

Summary

I. *The Preparation of the Experimental Mortars*

Starting with three cements as basic mixtures, (1) tricalcium silicate, (2) β-dicalcium silicate, (3) a mixture of (1) and (2) in equal proportions, 20% of each of these was substituted by tricalcium aluminate, $5CaO.3Al_2O_3$, monocalcium aluminate, $3CaO.5Al_2O_3$, dicalcium ferrite and $4CaO.Al_2O_3.Fe_2O_3$, respectively. The resulting mixtures as well as the pure aluminates and silicates (including γ-dicalcium silicate) were used as cements in the preparation of sand mortars. (Mainly mortars containing 1 part of the cement and 10 parts of standard sand by weight but also some 1:7½ and 1:5 mortars were used). Cements obtained by the substitution of 20% of the basic cements 1 and 3 above by the four calcium aluminates gave 1:10 mortars of good strength. Similar substitution by dicalcium ferrite and $4CaO.Al_2O_3.Fe_2O_3$ gave mortars which gained strength more slowly than was to be expected from the amount of tricalcium silicate present but continued to increase in strength for long periods of time. Mortars of β-dicalcium silicate and mixtures of the aluminates with this hardened rather slowly, while addition of 20% of dicalcium ferrite and $4CaO.Al_2O_3.Fe_2O_3$ gave a 1:10 mortar of very low strength at the end of seven weeks. Sand mortars made with tricalcium aluminate fell to pieces a short time after being exposed to pure water. Sand mortars made of γ-dicalcium silicate hardened extremely slowly but after 10 weeks in the molds they could be handled, gained slightly in strength on storing in water and much faster when stored in sulphate solutions.

II. *Action of the Sulphate Solutions on the Silicate Mortars*

All the mortars expand and disintegrate ultimately when exposed to solutions of magnesium sulphate, the rate of expansion increasing with the con-

centration of the solution. The order of decreasing rate of expansion of the 1:10 sand mortars was: β-dicalcium silicate, tricalcium silicate, γ-dicalcium silicate.

The expansion of the silicate mortars exposed to 0.15 *M* Na_2SO_4 and saturated calcium sulphate at the end of three years exposure differed only very slightly from that of similar mortars exposed to distilled water.

Exposure of the silicate mortars to solutions of sulphates at first causes a rapid increase in the tensile strength of the specimens. Later the strength falls off in the case of the mortar exposed to solutions of magnesium sulphate, but the decrease of tensile strength progresses much more slowly, and is less for corresponding linear expansion, than with mortars made from Portland cement. The tensile strength of 1:5 sand mortars stored in 0.15 *M* Na_2SO_4 was higher at the end of four years' exposure than that of similar mortars stored in distilled water.

III. *Action of the Sulphate Solutions on the Aluminate Mortars*

The 1:10 mortars expand and disintegrate very rapidly when exposed to any of the sulphate solutions. The rate of expansion decreases in the order: $5CaO.3Al_2O_3$, $CaO.Al_2O_3$, $3CaO.5Al_2O_3$.

The resistance of the mortars to sulphate action increases very rapidly with increased richness of mix, this being greatest for mortars made of monocalcium aluminate, so that these tend to become more resistant in the richer mixes than mortars made with the other aluminates.

The action of solutions of sodium sulphate proceeds mainly from the surface of the specimen inward, the disintegrated material sloughing off as the action progresses. The action of solutions of magnesium sulphate differs in this respect, the whole bar expanding rapidly and ultimately cracking to pieces.

IV. *Action of Sulphate Solutions on the Mortars made from Mixed Cements*

The rate of expansion of the 1:10 sand mortars containing the silicates with or without admixtures of the aluminates etc., when exposed to 0.15 *M* $MgSO_4$ decreases in the following order*:

80% C_3S + 20% C_3A (1)† > 80% C_3S + 20% C_5A_3 (1.1) > 80% C_3S + 20% CA; 40% C_3S + 40% β-C_2S + 20% C_3A (1.2) > 40% C_3S + 40% β-C_2S + 20% C_5A_3 (1.5) > 80% C_3S + 20% C_3A_5; 80% β-C_2S + 20% C_3A (1.6) > 40% C_3S + 40% β-C_2S + 20% CA (2.2) > 40% C_3S + 40% β-C_2S + 20% C_3A_5 (2.4) > Portland cement‡ (3) > C_3S + 20% C_4AF (5.5) > 80% β-C_2S + 20% C_5A_3 (9) > 80% C_3S + 20% C_2F (10) > 100% β-C_2S (15) > 40% C_3S + 40% β-C_2S + 20% C_4AF (17) > 100% C_3S (20) > 40% C_3S + 40% β-C_2S + 20% C_2F; 50% C_3S + 50% β-C_2S (39) > 80% β-C_2S + 20% CA (40) > 80% β-C_2S + 20% C_4AF (190)

The mortars made with the mixtures 80% β-C_2S + 20% C_3A_5 and 80% β-C_2S + 20% C_2F are not classified.

**Starting with the most rapidly expanding mortar and representing arbitrarily the time necessary for a linear expansion of 0.80% as unity, the numerals in brackets represent on the same scale the time required for the same linear expansion of the other mortars.*

†*Symbols used:* $C = CaO$; $S = SiO_2$; $A = Al_2O_3$; $F = Fe_2O_3$.

‡*Average value for eight commercial Portland cements manufactured in eight different plants.*

The mortars which expanded more rapidly than Portland cement mortars were similar to the latter in showing rapid loss in tensile strength during expansion. Those mortars which expanded more slowly than Portland cement mortars retained their strength remarkably well.

All the 1:10 mortars which expanded faster than Portland cement mortar in 0.15 *M* $MgSO_4$ also expanded faster in 0.15 *M* Na_2SO_4 and in saturated calcium sulphate. Of the mortars which expanded more slowly than the average for similar mortars of Portland cement only the one made with 80% C_3S + 20% C_4AF had expanded to disintegration at the end of three years' exposure to 0.15 *M* Na_2SO_4, the others having expanded less than 0.10% at that time. At the end of three years' exposure to the saturated solution of calcium sulphate the bars made with 80% C_3S + 20% C_4AF and 80% β-C_2S + 20% CA had reached an expansion of between 0.10 and 0.20%, the others having a linear expansion of less than 0.10%.

V. *Application to Hydraulic Cements*

1. All Portland cements high in lime and containing normal amounts of alumina have a low resistance to natural waters containing sulphates of magnesium, sodium or calcium. The higher the lime content of the cement (*i.e.* the higher the percentage of tricalcium silicate) the alumina remaining the same, the lower is the resistance of the cement to the action of the sulphates.

2. In the case of Portland cements high in lime (high in tricalcium silicate in comparison to the β-dicalcium silicate present) the resistance to the action of sulphates can be increased by decreasing the percentage of alumina (reducing the amount of tricalcium aluminate) or increasing the percentage of ferric oxide (changing tricalcium aluminate to $4CaO.Al_2O_3.Fe_2O_3$) or by both of these simultaneously. The resistance cannot be increased to equal that possessed by the pure silicate mixture present in the cement.

3. In the case of Portland cements low in lime (high in β-dicalcium silicate as compared with tricalcium silicate) the resistance to solutions of magnesium sulphate can be increased by the methods described in the preceding paragraph so that it becomes much higher than the resistance of the corresponding mixture of the pure silicates. While the resistance to solutions of sodium sulphate and calcium sulphate cannot be increased up to that of the silicate mixture, an extremely high resistance to these solutions can be obtained by this method.

4. In practice the limit to which the resistance can be increased by the methods in paragraph (3) is determined by the requirements of early strength since high later strengths are obtained with such mixtures.

5. High alumina cements, in order to have the highest resistance to the action of sulphate, should be of a composition which gives no aluminate higher in lime than monocalcium aluminate and the silica should be present as β-dicalcium silicate. The presence of a large quantity of the aluminate $3CaO.5Al_2O_3$ is probably not desirable on account of the lower strength and the action of water on this substance.

References

1. Bates, P. H. and Klein, A. A. Bur. Standards, Tech. Papers, 78. 1917.
2. Bogue, R. H. Ind. Eng. Chem. Anal. Ed. 1: 192-197. 1929.
3. Colony, R. J. Eng. News-Record, 86: 637. 1921.
4. Eckel, E. C. Cements, limes and plasters; their materials, manufacture and properties, 2d. ed. Wiley, New York. 1922.
5. Forsén, L. Zement, 19: 1130-1134, 1155-1160. 1930.
6. Hansen, W. C. Bur. Standards J. Research, 4: 55-72. 1930.
7. Hansen, W. C., Brownmiller, L. T. and Bogue, R. H. J. Am. Chem. Soc. 50: 396-406. 1928.
8. Lerch, W. and Bogue, R. H. J. Phys. Chem. 31: 1627-1646. 1927.
9. Lerch, W. and Bogue, R. H. Ind. Eng. Chem. Anal. Ed. 2: 296-298. 1930.
10. Miller, D. G. Public Roads, 5: 12-13. 1924.
11. Rankin, G. A. Ind. Eng. Chem. 7: 466-474. 1915.
12. Rankin, G. A. and Wright, F. E. Am. J. Sci. 39: 1-79. 1915.
13. Shelton, G. R. Ind. Eng. Chem. 17: 589-592. 1925.
14. Shelton, G. R. Ind. Eng. Chem. 17: 1267-1270. 1925.
15. Shelton, G. R. Ind. Eng. Chem. 18: 854-856. 1926.
16. Thorvaldson, T., Harris, R. H. and Wolochow, D. Ind. Eng. Chem. 17: 467-470. 1925.
17. Thorvaldson, T., Larmour, R. K. and Vigfusson, V. A. Eng. J. 10: 199-206. 1927.
18. Thorvaldson, T., Vigfusson, V. A. and Larmour, R. K. Trans. Roy. Soc. Can. III, 21: 295-310. 1927.
19. Thorvaldson, T., Wolochow, D. and Vigfusson, V. A. Can. J. Research, 1: 273-284. 1929.
20. White, A. H. Ind. Eng. Chem. 1: 5-11. 1909.

A CONVENIENT RING MOULD FOR RUBBER TESTING[1]

By D. F. Stedman[2]

Abstract

A mould is described which permits the direct moulding of rubber in the form of test rings, which for convenience may be made of the same radial dimensions as standard Schopper rings, while the thickness of the ring may be varied according to the amount of rubber inserted in the mould. These moulds are particularly useful when either the rubber or compounding ingredients are limited in amount, as they permit utilization of the whole of a sample, and tests may be obtained on quite small quantities.

It is very frequently necessary, especially in connection with chemical work on rubber, to make tensile or other tests on very small samples, when the quantities of either the rubber or filler available are definitely limited. The mould described has been found very convenient in such cases, as the whole of a sample may be utilized, and a fairly good test made on as little as 1 gm. of rubber, giving two rings, each nearly 1 mm. thick. To obtain two rings with a slab mould and Schopper dies requires 26 gm. of rubber, and only 11.4% of the material used is recovered in the form of test rings. Moulds of this type were used in tests on synthetic rubber recently described by Whitby and Katz (1).

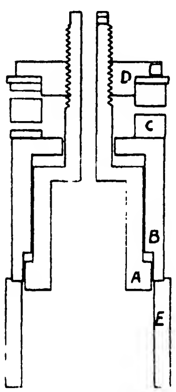

Fig. 1. *Ring mould.*

The mould is adapted for use in an oil or wax vulcanizing bath, or in an autoclave, but is not suited for use in a press. Those at present in use are turned from cold rolled mild steel.

In Fig. 1, the mould is shown in vertical cross section. The rubber is enclosed between the flange on *A*, and the recess of *B*. It will be seen that only the radial dimensions of the test ring, which are conveniently the same as those of the regular Schopper ring, are fixed by the mould, the vertical dimension varying according to the amount of rubber inserted.

A is bored out to about the same thickness of metal as *B*, resulting in an equal rate of heating, and preventing the opening of any appreciable crack between the parts. If any such separation occurs the space is at once filled with rubber, giving a permanent fin. A very slight fin is of course always obtained, but if the external faces of *A* and the internal faces of *B* are fitted as accurately as possible and highly finished, such leakage of rubber is practically negligible.

As the rubber is forced over these faces when the mould is opened care must be used to prevent scratches on the surfaces. This danger would be eliminated if the moulds were hardened and ground, but the cost of such moulds would be

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at least doubled. The moulds must also be cleaned very thoroughly after each use, and precautions taken against rusting, especially if chilled in cold water to stop the cure quickly.

In order to apply a definite and approximately known pressure to the rubber during cure a spring is used under the closing nut, giving about 750 lb. at 2 mm. compression. This spring must be designed very liberally in order to withstand the rather drastic treatment given by suddenly immersing in a vulcanizing bath. These springs have been increased in size considerably

FIG. 2. *The parts of the ring mould.*

since the photograph (Fig. 2) was taken, and the dimensions suggested are $2\frac{1}{4}$ turns made from a square bar $\frac{1}{2}$ by $\frac{3}{8}$ in. wound on a former $1\frac{1}{2}$ in. in diameter, the first and last turns being ground flat to give even pressure. The recessed ring E is used to open the mould by gentle pressure in a small hydraulic press. The spanner shown on the right of the photograph is used to open the nut, the head of which carries an appropriate hole.

In designing the mould to give a particular size of ring it must be noted that the ring obtained is about 1.3% smaller than the diameter of the mould. A tapered mandrel has been found very useful in measuring the internal diameter of rings, as the shrinkage varies a trifle with different stocks.

The specimen of rubber is milled in the usual way to give a sheet free from air bubbles, and a strip cut just long enough to wrap round A immediately above the flange. The ends are butted together and joined firmly. Such a joint has shown no tendency to weakness, but to remove all suspicion the sheet may be milled out to a thickness of about 1 mm. and wrapped round under tension several times, avoiding air pockets, but with the materials so far tested no difference was detectable in the results obtained by both methods. When inserting a specimen of rubber in the mold it has also been found advantageous to apply and release the pressure several times to allow the escape of air

It will be seen that the amount of rubber may be varied over wide limits and in fact for purposes of comparison rings have been made from 0.15 mm. to 7 mm. thick. Rings about 1 mm. thick give results only slightly lower than the maximum, while the optimum is at about 3 mm.

With these moulds a very slight "fin" is obtained on two edges, but this need not be removed completely as the dimensions are sufficiently uniform (to ± 0.01 mm.) that in most cases the fin need only be removed with a sharp pair of scissors from about 1 cm. of the ring. As the entire fin weighs only a few mg. it may be neglected in a tensile test, and if the same weight of rubber is used in each of a set of moulds, variations in dimensions are not usually sufficient to require correction.

Reference

1. WHITBY, G. S. and KATZ, M. Can. J. Research, 6: 398-408. 1932.

LIGNINS FROM CEREAL STRAWS

I. ISOLATION AND FRACTIONATION OF LIGNIN FROM OAT AND WHEAT STRAW[1]

By Léo Marion[2]

Abstract

The lignins isolated with methyl cellosolve (monomethyl ether of ethylene glycol) and concentrated hydrochloric acid, from both oat and wheat straw, have each been fractionated into five components by means of various solvents. The main fraction, soluble in acetone, which was isolated from oat-straw lignin has the same properties, methoxyl content and ultimate composition as the corresponding fraction of wheat-straw lignin. The components fractionated are compounds consisting of lignins combined with methyl cellosolve; lignins isolated by means of unmethylated solvents such as 1,4-dioxane have lower methoxyl values.

Plants belonging to families widely different from the Gramineae (Liliaceae, Compositae, Asclepiadaceae) gave rise through the identical process to lignins having different properties and composition than those isolated from straws.

Introduction

The quantitative isolation of lignin from cereal straws has been achieved hitherto only by the use of strong sulphuric acid (60-72%) or fuming hydrochloric acid, both methods destroying the non-ligneous part of the straw entirely. Dilute caustic soda, which has been used extensively, does not destroy the cellulosic material but removes less than half the lignin unless the extraction be run under pressure at higher temperatures. This method yields a product the solubility of which in organic liquids is negligible, and the purification of which is difficult and wasteful. It has been proposed to undertake a study of straw lignin with the purpose of investigating the properties of its sulphonic acids(sulphite liquors) especially in connection with tanning. To this end, the first object sought in the present work has been to isolate lignin in good yield from cereal straws by a process which, while removing the lignin in solution, would not destroy the cellulose and permit the recovery of the solvent. The action of 3% aqueous alkali at room temperature has not been found to give satisfactory results. A method which already had been applied by Fuchs (2) for the removal of lignin from spruce wood has been found remarkably suited to attain the object in view and also to yield a product having a relatively wide solubility range. As disclosed by this author the process involves the use of methyl cellosolve* containing 1% concentrated hydrochloric acid as catalyst. Neither catalyst nor solvent, however, is limited to those two substances and it is even advantageous to substitute 1,4-dioxane for methyl cellosolve as the former has a much lower boiling point and can therefore be reclaimed more readily. It has been found possible to separate the lignin obtained from both oat and wheat straw into fractions which are compared with the fractionation products of lignin isolated by Fuchs and Daur (3) from spruce wood.

[1] *Manuscript received April 12, 1932.*
Contribution from the National Research Laboratories, Ottawa, Canada.
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* *Monomethyl ether of ethylene glycol.*

Oat straw, to which the process was applied first, was subjected to preliminary extractions with a methanol-benzene mixture to dissolve fats and resins (4-6%), and with water to remove the components (11-13%) soluble in that medium. The dried straw, containing 23.8% lignin, was then heated with methyl cellosolve containing 1 cc. of concentrated hydrochloric acid per 1000 cc. The residue, after washing and drying, was not as strongly colored as the initial material but still retained the structure of straw. It had lost about 40% of its weight and still contained 13-13.5% lignin. After removal of most of the methyl cellosolve by distillation under reduced pressure, the lignin was precipitated from the residual solution by the addition of water. Pentosans were removed by mild hydrolysis and the product left then represented a yield of 7-8% of the weight of straw, or about one-third of the total lignin present. It will be noted that a quantity of material appreciably greater than the yield of lignin has been removed, partly with the methyl cellosolve solution and partly during the subsequent washing of the residue with water. This discrepancy between the yield of lignin and the loss in weight of the material used has been observed previously in the isolation of lignin from spruce wood by the same process (2) and also in a very similar method in which use is made of methyl cellosolve containing a trace of dry hydrogen chloride (4). The pentosans present in the straw (about 15%) do not make up the total loss but the carbohydrates originally combined with the liberated lignin may form most of the remainder.

The extraction of oat straw with a 3% aqueous solution of sodium hydroxide yields a lignin which, before purification, may amount to 10% of the weight of straw but is lowered considerably by the latter process. This alkali-lignin was found to consist of two portions, one soluble and the other insoluble in 95% alcohol. The soluble fraction was further differentiated by a higher methoxyl content (16.87%) than the insoluble (14.94%). That the fractions, however, are probably not homogeneous is indicated by the fact that the product obtained from oat straw by means of methyl cellosolve and hydrochloric acid, which it is proposed to term oat-straw methylin*, can be fractionated. Oat-straw methylin is a brown amorphous powder, soluble in dilute alkali hydroxides from which it is precipitated by mineral acids but not by carbon dioxide; it therefore probably contains one or more free carboxyl groups. The actions of several solvents reveal its heterogeneous nature. Ether dissolves 7.5% which is soluble also in ethyl acetate, acetone, methanol and methyl cellosolve. Ethyl acetate dissolves 7.0%, insoluble in ether, but soluble in acetone, methanol, methyl cellosolve and chloroform. Acetone then removes a large fraction (52-53%) insoluble in ether and ethyl acetate but soluble in acetone, methanol and methyl cellosolve. A further fraction (14.6%) dissolved by methanol is insoluble in ether, ethyl acetate and acetone but soluble in methanol and methyl cellosolve. The residue, 17.5%, insoluble in ether, ethyl acetate, acetone, and methanol, is dissolved by methyl cellosolve. If this methyl cellosolve solution of the residue is allowed to dry spontaneously

**Methylin is a word coined by W. Fuchs (3) to designate lignin which he had extracted from spruce-wood meal with methyl cellosolve containing concentrated hydrochloric acid.*

the lignin is left as a lustrous dark resinous layer displaying a strong sheen; the resin, however, shrinks on drying further and becomes brittle. It will be noted from Table I that the composition of each of these fractions differs from the others and that the greatest divergence occurs between the extreme fractions.

Spruce-wood methylin has been separated by Fuchs and Daur (3) into four fractions, three soluble in ether, acetone, and chloroform respectively and a residue soluble in methyl cellosolve. These fractions differ from the oat-straw methylins both in methoxyl values and in properties. The former are insoluble in sodium carbonate solution whereas those fractions of oat-straw methylin which are soluble in solvents not miscible with water can be extracted with a 5% sodium carbonate solution.

The oat-straw methylins, with the possible exception of the ether-soluble fraction, do not consist of lignin as present in the straw, but of compounds in which the solvent is combined with the lignin. This apparently accounts for the methoxyl content of oat-straw methylin being so much higher (about 21-22%) than that of alkali lignin isolated from the same source (about 15-16%). In fact, this is demonstrated by a comparison of oat-straw methylin with a lignin isolated by the same process in which a non-methylated solvent such as 1,4-dioxane was substituted for the methyl cellosolve. Dioxane-lignin obtained from oat straw contains 13-14.5% methoxyl, a value which, remembering that dioxane-lignin contains combined dioxane, is very close to that of alkali-lignin. The effect of dioxane on straw is very similar to that of methyl cellosolve, and there is a marked discrepancy between the loss of weight of the straw, 36-38%, and the yield of lignin, 4-6%. That the product isolated from plant materials by the use of solvents of the foregoing type is a compound of the lignin and the solvent, has also been the conclusion reached previously by Fuchs (2), by Hibbert and Marion (4) and more recently by Rassow and Gabriel (5).

Attempts to isolate lignin from straw by means of methyl-cellosolve containing 0.05% of its weight of dry hydrogen chloride according to the method used by Hibbert and Marion (4) yielded but a small quantity of lignin (about 0.5 – 3.0%) which did not seem to possess more attractive properties than the methylin isolated when concentrated hydrochloric acid was substituted for the dry gas.

Wheat straw, when treated exactly as the oat straw with methyl cellosolve and acid, was found to yield an appreciably larger quantity of methylin than the latter. The yield amounted to 11-12% of the weight of straw employed. After the treatment, the residual straw was thoroughly washed with water and dried. Its weight in numerous experiments was always found to lie between 60-65% of the initial weight, and 13-15% of this was still lignin. The residue retained the structure of the original straw but had a less intense yellow color. Methylin obtained from wheat straw has the same physical appearance as oat-straw methylin and possesses much the same properties. It is soluble in dilute aqueous alkali hydroxides, producing brown solutions from which it is precipitated by mineral acids although not by carbon dioxide, indicating the possible presence of free carboxylic groups. As in the case of oat straw it has been

possible by means of solvents to separate this methylin into five fractions: an ether-soluble fraction, about 8.2%, also soluble in ethyl acetate, acetone, methanol and methyl cellosolve; a further 9.3% soluble in ethyl acetate, acetone, methanol and methyl cellosolve, but insoluble in ether; a main fraction soluble in acetone, methanol and methyl cellosolve, but insoluble in ether and ethyl acetate and making up about 62.5% of the methylin; a small fraction, 4.2%, soluble in methanol and methyl cellosolve but insoluble in the first three solvents; and a residue soluble in methyl cellosolve, insoluble in the other solvents and amounting to 14.5% of the total. These results are compared in Table I with those obtained with oat-straw methylin.

TABLE I

FRACTIONS ISOLATED FROM OAT-STRAW AND WHEAT-STRAW METHYLINS

	Oat straw				Wheat straw			
Fraction	Per cent of total methylin	% C	% H	% OCH_3	Per cent of total methylin	% C	% H	% OCH_3
Ether	7.5	67.86	7.96	13.94	8.2	65.68	7.21	18.60
Ethyl acetate	7.0	62.04	6.06	22 48	9.3	60.14	5.72	20.80
Acetone	52.6	60.74	5.92	22.19	62.5	60.61	6.05	21.68
Methanol	14.6	61.02	6.08	20.96	4 2	60.82	5.93	21.34
Residue	17 5	60.77	5 90	19.24	14.5	60.79	5.61	17.25

NOTE:—*The figures tabulated here have been averaged from the values reported in the experimental part.*

Table I emphasizes the similarity in composition between the acetone fractions, obtained from oat-straw and wheat-straw methylins, and also between the methanol fractions. The members of any of the two pairs have practically the same carbon and hydrogen content and the same methoxyl values. They all possess similar properties: they are very readily soluble in dilute alkali hydroxides forming dark brown solutions from which carbon dioxide fails to precipitate them. Mineral acids added to these solutions precipitate the methylin as colloidal suspensions which are coagulated by hot water and then settle rapidly. It will be noted that the methanol-soluble fraction is obtained in much smaller yield from wheat-straw than from oat-straw methylin. The analytical figures of the two ethyl acetate-soluble fractions present a noticeable divergence and the difference in the case of the ether extracts is still wider so that comparison here precludes any idea of similarity.

On the basis of the foregoing results it can be concluded that both oat-straw and wheat-straw methylins are heterogeneous mixtures, each separable into at least five fractions possessing different physical properties and slightly different chemical compositions. Each mixture includes a predominating fraction soluble in acetone and similar to each other in physical properties and ultimate composition. Different smaller amounts of a fraction soluble in methanol are present which are also closely related. These two fractions

are in each case mixed with varying quantities of substances with markedly different elementary composition. It is probable that these latter various components are responsible for the differences noted between the two methylins as isolated from each source. As none of the products isolated have been obtained in a crystalline form there is no definite criterion and therefore no certainty concerning the homogeneity of any of the fractions. Furthermore, the analytical figures of the acetone-soluble and methanol-soluble fractions of oat-straw methylin and those of the corresponding fractions of wheat-straw methylin differ by a negligibly small margin, although these fractions are known to be different. Hence, until further evidence has been accumulated it is not justifiable to regard these fractions as identical.

The fraction isolated from wheat-straw methylin, through its solubility in ethyl acetate, was methylated in chloroform solution with methyl iodide and silver oxide, its methoxyl content being raised from 20.80% to 33.68%, a value which as expected is higher than that found (30.2%) for lignin isolated from straw with alkalies (1)*. It is also much higher than that found by Fuchs (2) for spruce-wood methylin after exhaustive methylation (26.5%). Spruce-wood lignin is therefore quite different from that isolated from oat straw or wheat straw.

Lignin has also been isolated from various other sources. Plant materials belonging to families offering widely different characteristics such as *Senecio retrorsus*, a South African species, *Zygadenus venenosus* and the seed hairs of *Asclepias cornuti* (Decaisne) have been treated by the foregoing methods of isolation. The lignins and methylins obtained have been found to be a great deal different in properties and composition from those isolated from either oat or wheat straw. Lignin was isolated from *S. retrorsus* by three methods: (a) methyl cellosolve containing concentrated hydrochloric acid, (b) the same solvent and dry hydrogen chloride, and also (c) 3% aqueous sodium hydroxide. The yield was best with the last method but was always very much lower than with the straws. If the residue from the alkaline treatment after washing and drying was heated with methyl cellosolve and concentrated hydrochloric acid, the methylin obtained consisted almost entirely of a substance insoluble in sodium hydroxide whereas the methylin isolated from the original material—before alkaline treatment—was soluble. Methylin, then, may possibly consist of the compounds formed between methyl cellosolve and the lignin which is usually removed from the plant by dilute alkalies at room temperature and ordinary pressure. The methylin obtained from *S. retrorsus* has a very low methoxyl content (3.6%) and it has colloidal properties different from those of the straw methylins.

Zygadenus venenosus, a species belonging to the Liliaceae gives rise to a methylin which on precipitation from an alkaline solution forms very tenacious colloidal suspensions which it was not possible to coagulate completely. Its methoxyl content (2.94%) is remarkably low.

This value (30.2%) has been calculated from the molecular weight and hydroxyl content given by Beckmann, Liesche and Lehmann for their alkali-lignin.

From the seed hairs of *A. cornuti* (Decaisne), the common milkweed, a methylin was isolated as an amorphous, light-brown powder. It dissolves readily in dilute sodium hydroxide to form a dark-brown solution from which it is quantitatively precipitated by a stream of carbon dioxide. It does not therefore contain any free carboxylic group. Its methoxyl content (24.56%) is much higher than that of oat-straw or wheat-straw methylin. The suspensions which it forms on precipitation from its solutions are very easily coagulated. The various lignins and methylins obtained besides the straw methylins are listed in Table II together with their methoxyl content.

TABLE II

LIGNINS AND METHYLINS ISOLATED FROM VARIOUS PLANTS

Material	Method of isolation	% C	% H	% OCH_3
Oat straw	HCl (sp. gr. 1.2)	60.95	6.06	11.75
Oat straw (sol. in alc.)	3% NaOH	59.30	5.62	16.87
Oat straw (insol. in alc.)	3% NaOH	59.58	5.60	14.94
Oat straw	Glycol-HCl	60.72	6.22	16.98
Senecio retrorsus	3% NaOH	53.56	6.52	3.61
Zygadenus venenosus	Me.cellosolve and conc. HCl	—	—	2.94
Maple bark	Me.cellosolve and conc. HCl	—	—	12.96
Common milkweed seed hairs	Me.cellosolve and conc. HCl	61.01	6.53	24.56

A methylin, as seen from Table II, was also obtained from the bark of a maple tree. This product after a great deal of purification was found to have a methoxyl content of 12.96%. Its colloidal properties are quite pronounced and its coagulation offered some difficulty.

Methylins behave as true colloids and form suspensions the coagulation of which is most readily obtained in a slightly acid medium, *i.e.*, having a very low pH. It is noteworthy that with the methylins investigated there seems to exist a relationship between the ease of coagulation and the methoxyl content of the product. The milkweed methylin which has the highest methoxyl content was coagulated with the greatest ease; oat-straw and wheat-straw methylins have a methoxyl content but slightly lower and were coagulated with about equal readiness. The methylins isolated from *S. retrorsus* and from *Z. venenosus*, which both have unusually low methoxyl contents, were never completely coagulated no matter what method was tried. On the other hand bark methylin, which has a methoxyl content (12.96%) intermediate between the latter and that of the straw or milkweed methylins, was coagulated with considerably less difficulty than the products obtained from *S. retrorsus* or *Z. venenosus* although the task was much less readily achieved than in the case of the straw methylins.

Experimental

Preparation of Methylin from Oat Straw

Dry oat straw free from chaff was ground and extracted first with a minimum boiling-point mixture of methanol and benzene (loss 4.5 − 5.5%) and then with water (loss 11.7 − 12.7%). The residue was dried, mixed with ten times its

weight of methyl cellosolve and 10 cc. of concentrated hydrochloric acid per 100 gm. of straw and heated on the water-bath for three to four hours. The mixture was filtered while hot on a Buchner and washed with hot methyl cellosolve until the washings were clear. After a subsequent thorough washing with water, the residue was weighed; it had lost from 39-44% of its original weight and still contained 13.4% lignin (determined with 72% H_2SO_4). The combined filtrate and washings were distilled under reduced pressure to remove most of the methyl cellosolve and the residual liquor poured into a large volume of water. The precipitated methylin was washed by decantation with hot water, heated for some 30 min. with 2% hydrochloric acid, to hydrolyze the pentosans, and then washed again with hot water. The methylin was then dissolved by the addition of sodium hydroxide, the solution filtered and precipitated by the addition of dilute hydrochloric acid. The precipitate was washed six to seven times by decantation with hot water (80° C.), poured onto a Buchner, washed with hot water and dried in a current of air at 60° C. Yield 7.5%. The results of several preparations are given in Table III.

TABLE III

ISOLATION OF METHYLIN FROM OAT STRAW

Expt. No.	Wt. of straw, gm.	Residual straw		Yield of methylin		Duration of experiment, hr.
		Gm.	% Loss in wt.	Gm.	%	
1	96	54.2	43.5	7.3	7.6	4
2	213	129	39.5	17.7	8.4	3
3	202	115	43.1	16.0	7.9	5
4	250	147	41.2	17.4	7.0	4
5	520	312	40.0	40.0	7.7	5
6	260	—	—	21.3	8.2	4
7	2114	1278	39.5	153.4	7.2	4

The isolated methylin is an amorphous brown powder, soluble in dilute sodium hydroxide from which solution it is precipitated by dilute mineral acids but not by a stream of carbon dioxide. It is also soluble in methyl cellosolve, glycol and pyridine.

Fractionation of Oat-straw Methylin

Oat-straw methylin was extracted in a Soxhlet with ether, ethyl acetate, acetone and methanol successively, the material between extractions being warmed for several hours in a stream of air to remove the former solvent.

To purify each fraction the solvent was removed by distillation, the residue dissolved in sodium hydroxide, and the solution filtered and acidified with dilute hydrochloric acid. The precipitated material was washed several times by decantation with hot water and finally filtered, washed on the filter and dried.

The ether-soluble fraction (6.8, 8.2%) is a light yellow amorphous powder soluble in ether, ethyl acetate, acetone, methanol and methyl cellosolve. Analysis:— C, 67.94, 67.79%; H, 7.84, 8.08%; OCH_3, 14.08, 13.79%.

Ethyl acetate-soluble fraction (yield varied from 7-13%): light-brown amorphous powder insoluble in ether but soluble in ethyl acetate, acetone, methanol and methyl cellosolve. Analysis:— C, 62.13, 61.94%; H, 5.99, 6.14%; OCH_3, 22.28, 22.69%.

Acetone-soluble fraction (the yield varied, being as low as 35% and as high as 53%): very light-brown powder, insoluble in ether and ethyl acetate but soluble in acetone, methanol and methyl cellosolve. Analysis:— C, 60.74, 60.74%; H, 5.90, 5.95%; OCH_3, 22.13, 22.25%.

Methanol-soluble fraction (10.6, 10.7%): light-brown amorphous powder insoluble in ether, ethyl acetate, and acetone but soluble in methanol and methyl cellosolve. Analysis:— C, 61.06, 60.97%; H, 6.19, 5.97%; OCH_3, 21.04, 20.88%.

The residue left (17.5, 22.2%) is a brown amorphous powder still soluble in methyl cellosolve. Analysis:— C, 60.79, 60.75%; H, 5.90, 5.89%; OCH_3, 19.26, 19.23%.

Preparation of Dioxane-lignin from Oat Straw

In the preparation of lignin by the foregoing procedure it is possible to replace methyl cellosolve by another solvent of lower boiling point so that the recovery of solvent is easier. 1, 4-Dioxane is very suitable for this purpose and exactly the same procedure can be used as with methyl cellosolve. The loss in weight of the straw is 38% and the yield of dioxane-lignin 5.2% calculated on the initial weight of straw. The product is a light-brown amorphous powder. Analysis:- C, 61.42, 61.49%; H, 5.57, 5.58%; OCH_3, 14.32, 14.28%. Anhydrous aluminium chloride can be substituted for the hydrochloric acid but the yield is lower (3.8%) although the loss in weight of the straw is of the same order as previously (36%) and the product has not the same analytical figures as the former, C, 63.09%; H, 5.42%; OCH_3, 12.73%.

Glycol Ether Lignin from Oat Straw

This product was obtained by means of methyl cellosolve containing 0.05% of its weight of dry hydrogen chloride. The procedure followed was that described by Hibbert and Marion (4) for the treatment of spruce wood. The yield was always very low, varying from 0.5 – 3%. Previous to precipitating the lignin from the methyl cellosolve solution, the latter was allowed to cool and a yellowish-white substance deposited which was filtered. This was found to be soluble in hot alcohol but practically insoluble in the cold; it was not further investigated.

Alkali-lignin from Oat Straw

The procedure used was that of Beckmann, Liesche and Lehmann (1) *i.e.*, the action of 3% sodium hydroxide at room temperature. The solution was separated from the residual straw by means of the basket centrifuge. The lignin precipitated with dilute mineral acid from the impure solution was heated with 2% hydrochloric acid to remove the pentosans. It was then redissolved in dilute sodium hydroxide—an appreciable portion remaining undissolved—the solution filtered through a layer of kieselguhr and exactly

neutralized with dilute hydrochloric acid which caused the precipitation of silicic acid. This was filtered out and the filtrate acidified, the precipitated lignin being washed several times by decantation with hot water (80° C.). It coagulated and settled rapidly; it was filtered, washed with hot water and again filtered. The product, an amorphous light-brown powder, was stirred into alcohol and allowed to stand for several days, the flask being shaken occasionally. The insoluble was then filtered, washed with alcohol and dried. The alcoholic filtrate was treated with charcoal, reduced to a small volume and poured into water, the precipitated alcohol-soluble lignin being filtered, washed and dried. The yield after this purification process was only 2%. Analysis:– (a) alcohol-soluble: C, 59.35, 59.25%; H, 5.72, 5.52%; OCH_3, 16.93, 16.81%. (b) alcohol-insoluble: C, 59.56, 59.61%; H, 5.59, 5.62%; OCH_3, 14.97, 14.91%.

Preparation of Methylin from Wheat Straw

The ground straw was extracted with a 50% by volume methanol-benzene mixture (11.4% loss) and with water. The dried residue was then treated with methyl cellosolve and concentrated hydrochloric acid in exactly the same way as in the case of oat straw. The product was also purified just as in the last instance. In Table IV are given the results of a series of extractions in which various conditions have been used.

TABLE IV

ISOLATION OF METHYLIN FROM WHEAT STRAW

Expt. No.	Wt. of straw, gm.	Me Cello-solve, cc.	Catalyst	Residual straw			Yield of methylin		Duration of of experiment, hr.
				Gm.	Loss in wt. %	Lignin content, %	Gm.	%	
1	206	1500	15 cc. conc. HCl	122	40.8	13.0	21.0	10.2	3.5
2	226	2000	20 cc. conc. HCl	130	42.5	—	24.8	10.9	4
3	42	440	4.5 cc. HAc	—	—	—	trace	—	2
4	194	1500	15 cc. conc. HCl	125	35.6	16.6	17.9	9.3	3.5
5	30	300	3 cc. H_3PO_4	—	—	14.4	—	—	2
6	203	2000	20 cc. conc. HCl	133	34.5	17.4	25.0	12.3	3
7	234	2000	20 cc. conc. HCl+5 cc. H_3PO_4	152	35.1	—	27.5	11.8	3.5
8	48	500	5 gm. hyd. $AlCl_3$	35	27.1	11.0	4.0	8.4	3
9	161	1600	15 gm. anhyd. $AlCl_3$	108	32.9	12.3	8.4	8.4	3.5

As will be noted in Experiments 8 and 9, the substitution of aluminium chloride, either hydrated or anhydrous, for the hydrochloric acid slightly lowered the yield. The use of acetic acid as catalyst was inefficient but phosphoric acid, although far less efficient than either hydrochloric acid or aluminium chloride, gave an appreciable yield. It had been hoped that phosphoric acid used along with the hydrochloric acid would facilitate coagulation of the precipitated methylin but it proved inefficient.

Fractionation of Wheat-straw Methylin

The fractionation was carried out exactly as for oat-straw methylin, the same solvents being used.

Ether-soluble fraction (9.9, 6.5%); a lemon-colored amorphous powder soluble in ether, ethyl acetate, acetone, methanol and methyl cellosolve. Analysis:– C, 65.70, 65.66%; H, 7.29, 7.13%; OCH_3, 18.61, 18.59%.

Ethyl acetate-soluble fraction (9.9, 9.1%); an amorphous, light-brown powder insoluble in ether but soluble in ethyl acetate, acetone, methanol and methyl cellosolve. Analysis:– C, 60.15, 60.12%; H, 5.75, 5.68%; OCH_3, 20.76, 20.85%.

Acetone-soluble fraction (62.6, 62.3%); a very light-brown amorphous powder insoluble in ether and ethyl acetate but soluble in acetone, methanol and methyl cellosolve. Analysis:– C, 60.53, 60.69%; H, 6.09, 6.01%; OCH_3, 21.81, 21.54%.

Methanol-soluble fraction (4.4, 4.0%); a light-brown amorphous powder insoluble in ether, ethyl acetate and acetone but soluble in methanol and methyl cellosolve. Analysis:– C, 60.64, 61.00%; H, 5.93, 5.93%; OCH_3, 21.24, 21.43%.

The residue left is a brown amorphous powder still soluble in methyl cellosolve. The methyl cellosolve solution on slow drying leaves a brittle, lustrous resinous mass. Analysis: C, 60.84, 60.74%; H, 5.63, 5.59 ; OCH_3, 17.03, 17.46%.

Methylation of Wheat-straw Methylin

The wheat-straw methylin soluble in ethyl acetate was dissolved in chloroform and the solution, mixed with equal weights of methyl iodide and silver oxide, was kept under reflux at 40° C. overnight. The mixture was filtered, the filtrate evaporated to dryness and the residue taken up in methyl cellosolve. After a treatment with charcoal the methyl cellosolve solution was poured into water and the precipitated product washed by decantation and on the filter with hot water and dried. It is a very light-brown amorphous powder. Analysis:– C, 62.27, 62.28%; H, 6.43, 6.43%; OCH_3, 33.84, 33.53%.

Preparation of Methylin from S. retrorsus

S. retrorsus is a shrub belonging to the Compositae and imported from South Africa. It was dried, ground, defatted with petroleum ether and extracted successively with methanol, benzene and water. The dried residue was then treated with methyl cellosolve and hydrochloric acid exactly as in the case of oat straw. As precipitated from its alkaline solution, however, the product was so fine that it ran through the filter. Neither heating nor the addition of aluminium sulphate could cause complete coagulation and settling, so the supernatant liquor containing very finely divided methylin in suspension was discarded. The loss in weight of the material was only 17.4%.

Alkali-lignin from S. retrorsus

The procedure followed here was exactly like that already described for oat straw. The product is a dark-brown powder soluble in alkali hydroxides. Analysis:– OCH_3, 2.89, 3.73 %. The residue left from the alkaline treatment,

after thorough washing and drying, gave rise, when heated with methyl cellosolve and hydrochloric acid, to a heterogeneous product the greater part of which was insoluble in dilute sodium hydroxide; it was not further examined.

Methylin from Z. venenosus

This plant which belongs to the Liliaceae was treated exactly as *S. retrorsus*. It suffered a loss in weight of 24% and yielded 1.7% of methylin. This product is a very dark-brown amorphous powder which throughout the procedure remained in a very finely divided state, which accounts for the very low yield obtained. Analysis:– OCH_3, 2.87, 3.00%.

Isolation of Methylin from Maple Bark

The dry bark of an old tree was crushed and ground to a powder. It was then defatted with petroleum ether (2%), extracted with methanol-benzene mixture (loss, 6.1%) and then with water. The product, which contained 35.1% lignin (determined by means of 72% H_2SO_4) was treated with methyl cellosolve and hydrochloric acid according to the procedure already described. This methylin (yield 9.5%) is a reddish amorphous powder, soluble in sodium hydroxide from which solution it is precipitated by mineral acids. Analysis:– OCH_3, 12.86, 13.07%. The product had to be washed with a large quantity of hot water in order to remove completely a soluble red substance.

Methylin from the Seed Hairs of the Common Milkweed

The seed hairs from the common milkweed (*A. cornuti*) were extracted with petroleum ether, a mixture of methanol-benzene and finally with water prior to being treated with methyl cellosolve and hydrochloric acid. The product, a light-brown amorphous powder, is soluble in dilute sodium hydroxide from which solution it is completely precipitated by a stream of carbon dioxide. Analysis:– OCH_3, 24.57, 24.54%.

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References

1. Bechmann, E., Liesche, O. and Lehmann, F. Z. angew. Chem. 34: 285-288. 1921.
2. Fuchs, W. Ber. 62: 2125-2132. 1929.
3. Fuchs, W. and Daur, R. Cellulosechemie, 12: 103-110. 1931.
4. Hibbert, H. and Marion, L. Can. J. Research, 2: 364-375. 1930.
5. Rassow, B. and Gabriel, H. Cellulosechemie, 12: 249-254. 1931.
6. Schorger, A. W. Ind. Eng. Chem. 17: 642. 1925.

THE DETERMINATION OF MOISTURE IN HONEY[1]

By H. D. Chataway[2]

Abstract

The relationships between refractive index and moisture content of honey and between viscosity and moisture content have been quantitatively investigated, and as a result it has been found possible to utilize each of these properties for the determination of the moisture content. The requisite tables have been drawn up and details of the methods given. The degree of accuracy attainable is equal to that of the standard A.O.A.C. method, while the procedure is in both cases simpler and more rapid.

Introduction

In the analysis of honey the determination of moisture content has always occupied a foremost place. Not only does moisture determine the consistency and to that extent the general quality of the honey, but it is also of importance in the question of fermentation (2, 5). Nevertheless the methods employed have long been regarded as unsatisfactory.

On the one hand there is the standard A.O.A.C. method which, on account of the time and attention to detail required, is suitable only for theoretical work. Moreover the results so obtained stand alone, unsupported by other evidence, and in consequence doubt often arises as to their accuracy (3). On the other hand practical men resort almost entirely to some form of honey hydrometer, and grade honey according to degrees Baumé. Because of the high viscosity of honey the determinations are usually made between 120° and 140° F., (49° C.—60° C.), but even then they are of very uncertain accuracy.

Intermediate between the absolute and the hydrometer methods is that based on refractive indices. Unfortunately the only tables available for the conversion of refractive index readings into per cent moisture have been those to be found in sugar handbooks. When applied to honeys these give results which are nearly 2% too high in moisture, in all probability because the refractive indices of cane sugar and of invert sugar differ considerably (4). As a result, the value of the refractometric method has been unduly discredited, in addition to the fact that its general use has been discouraged by considerations of expense.

The object of the present investigation was to discover, if possible, a method which should be both rapid and accurate and which should not involve the use of an expensive instrument. From the outset the hope was entertained that this might be accomplished by means of viscosity measurements. Since it was evident that a considerable number of honey samples would have to be analyzed for moisture by the standard method, a somewhat lengthy procedure, it was decided to carry on a study of the refractive index method at the same time, making use of the same samples.

Both lines of work have proved successful. The viscosity of honey has been found to be very sensitive to moisture content, a difference of 0.1% in the

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latter giving rise to differences in viscosity ranging from 4 to 6%. The decrease in viscosity per unit increase in moisture content depends upon the temperature and also upon the moisture content itself. However, tables have been drawn up which take full account of these factors, and make it possible to calculate rapidly the moisture content of any honey given the time of fall of a standard steel ball at any observed room temperature.

The refractive index of honey is likewise found to give an accurate indication of moisture content. The ordinary sugar tables, however, must not be used. Instead a special honey table has been drawn up on the basis of the present work, and by its use correct moisture figures are obtained.

Experimental

Preliminary Viscosity and Refractive Index Measurements

The arbitrary viscosities at 25° C. of a limited number of honey samples were determined by the falling-ball method, while at the same time the refractive indices were taken. The refractive indices were plotted against the logarithms of the viscosities, and both refractive index and log viscosity were plotted against the absolute moistures as determined by the standard A.O.A.C. method. The curves obtained were almost straight lines, the individual points lying close to the lines. Moreover the correspondence was as good in the log viscosity-refractive index curve as in the other two curves. Since these preliminary experiments proved satisfactory a systematic study of the viscosity and refractive index properties of honey was undertaken.

Temperature Corrections Determined

(*a*) *Correction of refractometer reading.* The first step was to determine temperature corrections in order that readings might be taken at room temperature and the necessity of accurate and tedious temperature control eliminated. The correction per degree Centigrade proved to be independent of the temperature and of the moisture content of the honey. Thus, using the Pulfrich refractometer, in the case of two honeys having moisture contents of 18.2 and 14.5%, the change in refractive index between 15° C. and 25° C. was accurately linear and equal to −.000232 and −.000223 per degree respectively. Within experimental error, therefore, the temperature correction may be taken as −.00023 per degree Centigrade.

(*b*) *Corrections of viscosity.* In the case of the viscosity correction, however, much greater difficulty was encountered, for it was found that the correction per degree Centigrade varied slightly, not only with the temperature, but also with the moisture content of the honey. As a starting point, therefore, the viscosity-temperature relationships of a series of representative honeys were carefully determined.

For this purpose a jacketed glass tube was used, about 18 cm. long and 16 mm. in internal diameter (Fig. 1). A small glass tube, just slightly larger in diameter than the steel ball-bearing the time of fall of which was to be determined,

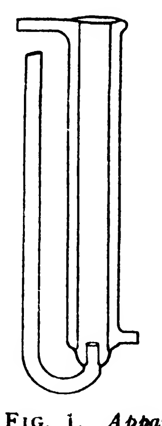

FIG. 1. *Apparatus used for determining viscosities of honey at different temperatures.*

projected for about 8 mm. of its length within the closed end of the jacketed tube. In order to facilitate washing and thorough drying, the external end of this small tube was closed with rubber tubing and a glass stopper.

In carrying out a determination the apparatus was filled with honey, the steel ball introduced and the open ends corked. The temperature of the honey was then carefully adjusted by passing through the jacket a constant stream of water of the exact temperature desired. At the same time the steel ball was guided into the small tube through which it moved slowly due to the fact that the tube was only slightly larger in diameter than the ball. The apparatus was then inverted and the delay which occurred before the ball left the small tube allowed all final adjustments of temperature, position of the tube, etc., to be made without haste. In certain cases where more time was needed, the ball was held at the bend in the small tube by means of a magnet suspended above it. The time of fall of the ball between two marks on the tube, 14 cm. apart, was then noted, using a stopwatch. Table I shows a typical set of results obtained for one honey.

TABLE I

TEMPERATURE-VISCOSITY READINGS FOR ONE HONEY

Temp., °C.	Time of fall, sec.	Temp., °C.	Time of fall, sec.
15	150.0	25	33.8
15	148.6	25	34.0
18	93.4	28	22.7
18	91.3	28	22.4
18	92 4	30	17.5
20	67.0	30	17.1
20	67 5	32	13 4
22	50.6	32	13.8
22	50 6	35	9.2
25	33 9	35	9.2

Similar sets of determinations were made for five other honeys of representative moisture contents. On plotting log viscosity against log absolute temperature, the graphs were found to be markedly non linear. An endeavor was then made to find such a function $F(t,\ V_{25})$ that log viscosity plotted against log $F(t,\ V_{25})$ should be substantially linear, so that

$$\log V_t = M \log F(t,\ V_{25}). \qquad (1)$$

It was found that $F(t,\ V_{25}) = 48 + t - .24 V_{25}$ fulfilled this condition very satisfactorily.

In any given case, over the range $t = 15°$ C. to $t = 30°$ C. the slopes of the lines were

$$M = \frac{\log V_{15} - \log V_{30}}{\log\ (48+15-.24 V_{25}) - \log\ (48+30-24 V_{25})} \qquad (2)$$

$$M = \frac{\log V_{15} - \log V_{30}}{\log \left[\dfrac{63-.24\ V_{25}}{78-.24\ V_{25}} \right]} \qquad (3)$$

It was then found that, with a high degree of accuracy, for all the honeys

$$\log V_{15} - \log V_{30} = \frac{2.2 + \log V_{25}}{4}$$

whence

$$M=\frac{2.2+\log V_{25}}{4\log\left[\dfrac{63-.24\ V_{25}}{78-.24\ V_{25}}\right]} \tag{4}$$

It will now be seen that, from (1), (4),

$$\log V_{25}-\log V_t=\mathrm{M}\left[\log(48+25-.24\ V_{25})-\log(48+t-.24\ V_{25})\right]$$

whence

$$\log\left(\frac{V_{25}}{V_t}\right)=\frac{(2.2+\log V_{25})}{4\log\left(\dfrac{63-.24\,V_{25}}{78-.24\,V_{25}}\right)}\log\frac{(73-.24\ V_{25})}{(t+48-.24\,V_{25})} \tag{5}$$

from which the values of $\frac{V_{25}}{V_t}$ were computed.

This however, gives $\frac{V_{25}}{V_t}$ as a function of V_{25} and not as a function of V_t. Therefore, for a series of honeys, V_t was plotted as a function of t, and also the ratio $\frac{V_{25}}{V_t}$ as a function of t.

This gave two families of curves, from which, by interpolation of corresponding points between corresponding curves, $\frac{V_{25}}{V_t}$ was tabulated as a function of V_t, thus obtaining the desired correction factors.

Lastly, in order to eliminate any errors that might have crept in, the correction factors so obtained were themselves plotted and the final values were taken from smoothed curves. This, however made little difference in their values.

The final figures are given in Table II. In practice it is only necessary to multiply the observed time of fall at any temperature by the proper factor for that time and for that temperature, as found from this table, in order to obtain the time that would be required for the ball to fall the same distance through the honey at a temperature of 25° C.

As the preliminary experiments indicated, the logarithms of the time of fall, and of the refractive index are nearly proportional to the moisture content. It remained, therefore, to draw up tables showing the exact relationship of viscosity and refractive index, (both at a standard temperature—25° C.), to per cent of moisture.

Analysis of Samples

(*a*) *Methods*. As a basis for these tables some 60 honeys, collected from all parts of Canada and also from the United States, were examined. The viscosity, refractive index, and moisture content were determined at one time for each honey, in order to avoid any error due to possible changes in the honeys.

The procedure followed for the determination of viscosity was as simple as possible, the aim being to carry out the test exactly as it would be done in routine work, except that check determinations were made in all cases.

TABLE II
Temperature correction factors for honey viscosity measurements

Time of fall, sec.	Temperature, °C.																											Time of fall, sec.
	15.0	15.2	15.4	15.6	15.8	16.0	16.2	16.4	16.6	16.8	17.0	17.2	17.4	17.6	17.8	18.0	18.2	18.4	18.6	18.8	19.0	19.2	19.4	19.6	19.8	20.0	20.2	
10.0																								.536	.550	.564	.577	10.0
12.5													.414	.423	.432	.442	.451	.462	.472	.484	.495	.507	.517	.531	.544	.557	.571	12.5
15.0	.305	.313	.322	.330	.338	.346	.354	.363	.373	.382	.390	.399	.408	.417	.427	.437	.447	.458	.468	.480	.491	.503	.515	.527	.540	.552	.565	15.0
17.5	.301	.310	.317	.325	.333	.341	.351	.360	.369	.377	.386	.395	.404	.412	.423	.433	.442	.453	.464	.475	.486	.497	.510	.520	.534	.545	.559	17.5
20.0	.298	.306	.314	.322	.330	.338	.347	.356	.365	.373	.383	.391	.401	.408	.419	.429	.439	.449	.460	.470	.482	.493	.505	.515	.530	.540	.554	20.0
25.0	.293	.298	.306	.313	.321	.329	.338	.348	.358	.367	.375	.381	.391	.399	.409	.420	.429	.439	.448	.458	.472	.483	.493	.506	.521	.531	.546	25.0
30.0	.287	.292	.300	.307	.316	.323	.331	.341	.350	.358	.366	.373	.383	.392	.401	.413	.422	.433	.441	.452	.465	.476	.486	.499	.513	.525	.539	30.0
35.0	.282	.288	.295	.302	.311	.317	.324	.334	.343	.351	.358	.366	.376	.386	.395	.407	.416	.428	.435	.446	.458	.470	.480	.493	.507	.519	.533	35.0
40.0	.277	.283	.291	.298	.306	.312	.319	.329	.337	.345	.354	.360	.371	.380	.389	.401	.410	.422	.430	.441	.452	.464	.475	.488	.501	.513	.528	40.0
50.0	.269	.275	.282	.289	.298	.303	.310	.320	.327	.336	.344	.350	.361	.371	.380	.391	.400	.413	.421	.433	.443	.455	.467	.478	.492	.504	.519	50.0
60.0	.262	.268	.275	.282	.290	.296	.303	.313	.319	.328	.336	.343	.353	.363	.372	.383	.392	.405	.413	.425	.435	.448	.459	.471	.484	.497	.511	60.0
70.0	.256	.263	.269	.276	.284	.290	.297	.305	.312	.321	.329	.337	.347	.357	.366	.376	.386	.398	.407	.418	.428	.441	.453	.466	.478	.491	.509	70.0
80.0	.251	.257	.264	.271	.278	.284	.291	.300	.307	.316	.324	.331	.341	.351	.360	.369	.380	.392	.401	.412	.423	.435	.447	.460	.473	.485	.500	80.0
90.0	.246	.253	.259	.266	.273	.279	.287	.295	.302	.311	.319	.326	.336	.346	.355	.364	.375	.387	.397	.407	.418	.430	.442	.456	.468	.481	.495	90.0
100.0	.242	.248	.255	.262	.268	.275	.283	.291	.297	.306	.314	.322	.332	.342	.351	.360	.371	.382	.392	.403	.414	.426	.438	.452	.464	.477	.491	100.0
110.0	.239	.245	.251	.257	.264	.271	.279	.287	.294	.302	.310	.318	.328	.338	.347	.356	.367	.378	.388	.399	.410	.422	.434	.448	.461	.473	.488	110.0
120.0	.235	.241	.247	.254	.261	.268	.275	.284	.291	.299	.307	.315	.325	.334	.344	.353	.364	.375	.385	.396	.407	.419	.431	.445	.457	.470	.485	120.0
140.0	.229	.236	.241	.248	.255	.262	.269	.278	.285	.293	.301	.309	.319	.328	.338	.347	.356	.368	.379	.390	.401	.413	.425	.439	.452	.465	.479	140.0
160.0	.225	.231	.236	.243	.250	.257	.264	.273	.280	.288	.296	.304	.314	.323	.333	.342	.353	.363	.374	.385	.397	.408	.420	.434	.448	.460	.475	160.0
180.0	.221	.226	.232	.239	.246	.253	.260	.268	.276	.283	.292	.300	.309	.318	.328	.338	.348	.359	.370	.381	.393	.404	.416	.430	.444	.456	.471	180.0
200.0	.217	.223	.228	.235	.242	.249	.256	.264	.272	.280	.288	.297	.306	.315	.325	.334	.344	.355	.366	.378	.389	.400	.413	.426	.440	.453	.468	200.0
240.0	.212	.217	.222	.229	.236	.243	.250	.258	.266	.274	.282	.290	.299	.308	.318	.327	.338	.348	.360	.371	.383	.394	.407	.419	.436	.448	.462	240.0
280.0	.207	.212	.218	.224	.231	.238	.245	.252	.260	.269	.277	.284	.294	.303	.312	.321	.332	.343	.354	.366	.377	.389	.402	.414	.428	.443	.458	280.0
320.0	.202	.208	.213	.220	.224	.233	.241	.248	.256	.264	.273	.279	.289	.298	.307	.316	.328	.339	.349	.362	.372	.385	.398	.411	.424	.439	.454	320.0
380.0	.197	.203	.208	.214	.220	.227	.234	.242	.250	.257	.267	.274	.282	.291	.301	.310	.322	.333	.344	.356	.368	.380	.394	.406	.421	.436	.452	380.0
440.0	.192	.197	.203	.209	.215	.223	.229	.236	.244	.252	.261	.269	.277	.286	.296	.306	.317	.328	.340	.352	.364	.377	.391	.403	.418	.435	.449	440.0
500.0	.187	.193	.199	.205	.210	.217	.224	.231	.239	.247	.257	.264	.272	.282	.292	.302	.313	.324	.337	.348	.361	.374	.389	.402	.415	.433	.448	500.0
600.0	.180	.186	.193	.199	.205	.211	.218	.224	.233	.241	.250	.257	.266	.276	.286	.297	.308	.320	.332	.344	.357	.369	.384	.398	.413	.432	.447	600.0
700.0	.175	.181	.187	.194	.200	.206	.212	.219	.227	.235	.243	.251	.260	.271	.282	.293	.304	.316	.329	.341	.354	.367	.382	.397	.412	.431	.446	700.0
800.0	.168	.175	.182	.189	.195	.201	.207	.214	.221	.228	.237	.246	.255	.267	.276	.289	.301	.314	.327	.339	.352	.365	.382	.396	.412	.430	.446	800.0
1000.0	.161	.167	.174	.181	.186	.192	.199	.206	.212	.221	.230	.239	.248	.261	.272	.284	.297	.310	.324	.337	.350	.364	.382					1000.0
1200.0	.154	.160	.167	.173	.180	.186	.191	.198	.207	.215	.225	.235	.245	.258	.271	.283	.296	.310										1200.0
1400.0	.151	.157	.163	.171	.177	.183	.189	.196	.204	.214	.224																	1400.0

TABLE II—*Continued*

Time of fall, sec.	Temperature in °C. 20.4	20.6	20.8	21.0	21.2	21.4	21.6	21.8	22.0	22.2	22.4	22.6	22.8	23.0	23.2	23.4	23.6	23.8	24.0	24.2	24.4	24.6	24.8	25.0	25.2	25.4	Time of fall, sec.
2.5																									1.022	1.045	2.5
5.0																		.875	.894	.915	.935	.955	.977	1.000	1.023	1.046	5.0
7.5							.688	.704	.718	.735	.750	.768	.784	.801	.818	.833	.851	.872	.892	.913	.933	.955	.976	1.000	1.024	1.047	7.5
10.0	.590	.605	.617	.633	.647	.662	.677	.694	.709	.725	.742	.758	.777	.793	.811	.829	.848	.869	.889	.911	.932	.954	.976	1.000	1.024	1.048	10.0
12.5	.584	.598	.612	.626	.641	.657	.672	.688	.704	.721	.737	.754	.771	.789	.808	.826	.845	.866	.887	.909	.931	.953	.975	1.000	1.025	1.049	12.5
15.0	.579	.593	.607	.622	.636	.651	.667	.682	.699	.715	.733	.751	.766	.785	.802	.823	.843	.863	.885	.907	.930	.952	.975	1.000	1.025	1.050	15.0
17.5	.574	.586	.600	.615	.629	.644	.660	.676	.693	.709	.727	.746	.762	.782	.799	.820	.840	.862	.883	.906	.928	.951	.975	1.000	1.026	1.051	17.5
20.0	.569	.582	.595	.610	.625	.640	.655	.671	.688	.705	.724	.743	.759	.779	.797	.818	.838	.860	.882	.904	.927	.950	.974	1.000	1.026	1.051	20.0
25.0	.561	.574	.587	.600	.617	.632	.648	.664	.681	.698	.717	.736	.753	.773	.792	.813	.834	.856	.878	.901	.926	.949	.974	1.000	1.027	1.053	25.0
30.0	.554	.566	.580	.594	.611	.626	.641	.658	.676	.693	.711	.730	.748	.769	.788	.809	.831	.853	.876	.899	.924	.948	.973	1.000	1.027	1.054	30.0
35.0	.548	.560	.574	.588	.605	.620	.636	.653	.671	.688	.707	.726	.744	.765	.785	.806	.828	.850	.874	.897	.922	.947	.973	1.000	1.028	1.055	35.0
40.0	.542	.556	.569	.584	.600	.616	.631	.649	.667	.684	.702	.722	.740	.762	.782	.803	.825	.848	.871	.895	.921	.946	.972	1.000	1.028	1.056	40.0
50.0	.532	.547	.561	.576	.592	.608	.624	.642	.660	.678	.695	.715	.734	.756	.777	.798	.820	.845	.868	.892	.918	.944	.971	1.000	1.029	1.057	50.0
60.0	.524	.540	.554	.569	.585	.601	.617	.635	.654	.672	.689	.710	.730	.752	.773	.795	.817	.842	.866	.889	.916	.943	.971	1.000	1.030	1.058	60.0
70.0	.518	.534	.548	.563	.579	.596	.612	.630	.649	.667	.685	.706	.726	.748	.770	.792	.814	.839	.863	.888	.915	.942	.970	1.000	1.030	1.060	70.0
80.0	.513	.528	.543	.559	.575	.591	.607	.626	.645	.664	.681	.702	.722	.745	.767	.789	.812	.838	.862	.886	.914	.941	.970	1.000	1.031	1.061	80.0
90.0	.509	.524	.538	.555	.571	.587	.603	.622	.642	.660	.678	.699	.719	.742	.764	.787	.810	.836	.860	.885	.913	.940	.970	1.000	1.031	1.062	90.0
100.0	.505	.520	.535	.551	.567	.583	.600	.619	.638	.657	.676	.697	.717	.740	.763	.785	.808	.835	.859	.885	.912	.940	.969	1.000	1.031	1.062	100.0
110.0	.502	.516	.531	.547	.563	.580	.598	.616	.636	.655	.674	.695	.715	.738	.761	.784	.807	.834	.858	.884	.911	.939	.969	1.000	1.032	1.063	110.0
120.0	.499	.513	.528	.544	.561	.577	.595	.613	.633	.652	.672	.693	.713	.736	.760	.783	.806	.833	.857	.884	.910	.939	.969	1.000	1.032	1.063	120.0
140.0	.493	.508	.523	.540	.555	.573	.591	.609	.629	.649	.669	.690	.710	.734	.758	.781	.804	.832	.856	.883	.910	.938	.970	1.000	1.032	1.063	140.0
160.0	.489	.504	.519	.536	.551	.569	.588	.605	.626	.645	.666	.688	.709	.732	.757	.780	.803	.831	.856	.883	.910	.938	.970	1.000	1.031	1.062	160.0
180.0	.485	.500	.515	.532	.548	.566	.586	.603	.624	.643	.664	.687	.707	.731	.757	.779	.803	.830	.856	.883	.909	.938	.970	1.000	1.030	1.062	180.0
200.0	.482	.497	.512	.530	.546	.564	.584	.601	.622	.641	.663	.686	.707	.730	.756	.779	.803	.830	.856	.884	.908	.938	.970	1.000	1.029	1.060	200.0
240.0	.476	.492	.508	.526	.542	.560	.581	.599	.620	.638	.661	.685	.707	.730	.757	.780	.804	.831	.857	.884	.909	.938	.970	1.000	1.026	1.057	240.0
280.0	.472	.488	.504	.524	.540	.558	.580	.599	.618	.637	.662	.686	.710	.734	.761	.784	.809	.833	.858	.884	.909	.939	.971	1.000	1.022	1.053	280.0
320.0	.470	.487	.502	.522	.539	.558	.580	.600	.621	.639	.664	.691	.716	.741	.767	.790	.816	.838	.861	.886	.911	.941	.972	1.000	1.017	1.048	320.0
380.0	.467	.485	.502	.523	.540	.559	.583	.604	.627	.642	.674	.704	.734	.760	.784	.803	.831	.848	.868	.890	.914	.945	.973	1.000			380.0
440.0	.465	.484	.501	.524	.542	.563	.589	.612	.637	.663	.692	.729	.762	.789	.812	.828	.848										440.0
500.0	.464	.483	.502	.526	.545	.569	.592	.623	.652	.682	.718	.761															500.0
600.0	.463	.483	.504	.532	.554	.584	.616	.649																			600.0
700.0	.464	.484	.509	.541	.569																						700.0
800.0	.465	.486																									800.0

TABLE II—*Continued*

Time of fall, sec.	Temperature in °C.																							Time of fall, sec.
	25.6	25.8	26.0	26.2	26.4	26.6	26.8	27.0	27.2	27.4	27.6	27.8	28.0	28.2	28.4	28.6	28.8	29.0	29.2	29.4	29.6	29.8	30.0	
2.5	1.070	1.080	1.110	1.134	1.158	1.183	1.206	1.232	1.257	1.283	1.310	1.340	1.367	1.398	1.430	1.460	1.493	1.526	1.560	1.597	1.633	1.670	1.706	2.5
5.0	1.072	1.088	1.113	1.140	1.164	1.191	1.219	1.248	1.276	1.305	1.335	1.363	1.395	1.427	1.460	1.493	1.528	1.564	1.600	1.637	1.674	1.711	1.748	5.0
7.5	1.073	1.093	1.119	1.146	1.172	1.200	1.229	1.260	1.289	1.320	1.351	1.382	1.416	1.453	1.483	1.520	1.558	1.594	1.633	1.675	1.716	1.756	1.796	7.5
10.0	1.075	1.098	1.124	1.151	1.180	1.208	1.239	1.270	1.301	1.330	1.365	1.398	1.434	1.471	1.507	1.544	1.582	1.622	1.663	1.707	1.749	1.795	1.839	10.0
12.5	1.076	1.101	1.128	1.155	1.186	1.214	1.246	1.277	1.310	1.343	1.377	1.412	1.450	1.488	1.524	1.564	1.606	1.649	1.688	1.733	1.779	1.826	1.875	12.5
15.0	1.078	1.103	1.132	1.159	1.191	1.220	1.252	1.283	1.318	1.352	1.387	1.423	1.462	1.502	1.540	1.580	1.622	1.668	1.708	1.754	1.806	1.853	1.901	15.0
17.5	1.079	1.106	1.135	1.163	1.195	1.225	1.258	1.289	1.324	1.360	1.396	1.433	1.473	1.514	1.554	1.595	1.637	1.684	1.725	1.772	1.825	1.875	1.926	17.5
20.0	1.080	1.108	1.137	1.166	1.199	1.229	1.263	1.294	1.330	1.367	1.404	1.442	1.482	1.525	1.565	1.607	1.649	1.698	1.740	1.787	1.842	1.895	1.945	20.0
25.0	1.082	1.111	1.142	1.171	1.204	1.236	1.271	1.304	1.341	1.379	1.417	1.457	1.498	1.542	1.583	1.626	1.671	1.720	1.765	1.813	1.868	1.926	1.976	25.0
30.0	1.083	1.114	1.145	1.175	1.209	1.241	1.277	1.312	1.350	1.388	1.428	1.468	1.510	1.555	1.597	1.643	1.688	1.737	1.785	1.834	1.891	1.946	2.000	30.0
35.0	1.085	1.116	1.148	1.179	1.213	1.246	1.283	1.317	1.358	1.397	1.436	1.478	1.521	1.566	1.609	1.656	1.701	1.751	1.801	1.851	1.908	1.966	2.018	35.0
40.0	1.086	1.118	1.150	1.182	1.217	1.250	1.288	1.324	1.364	1.403	1.443	1.486	1.530	1.574	1.618	1.668	1.712	1.763	1.814	1.866	1.921	1.980	2.034	40.0
50.0	1.088	1.121	1.153	1.187	1.222	1.256	1.295	1.331	1.373	1.413	1.454	1.499	1.543	1.588	1.633	1.684	1.730	1.780	1.834	1.888	1.940	1.998	2.054	50.0
60.0	1.090	1.123	1.156	1.191	1.226	1.261	1.300	1.338	1.379	1.421	1.462	1.507	1.552	1.597	1.643	1.693	1.741	1.793	1.846	1.897	1.950	2.003	2.059	60.0
70.0	1.092	1.124	1.158	1.194	1.229	1.264	1.304	1.343	1.384	1.426	1.468	1.512	1.557	1.602	1.647	1.696	1.743	1.796	1.845	1.894	1.948	1.997	2.052	70.0
80.0	1.092	1.126	1.160	1.196	1.231	1.267	1.307	1.347	1.387	1.428	1.471	1.514	1.558	1.602	1.647	1.694	1.738	1.789	1.839	1.885	1.936	1.984	2.033	80.0
90.0	1.093	1.126	1.161	1.197	1.233	1.269	1.308	1.349	1.388	1.429	1.471	1.513	1.557	1.600	1.644	1.689	1.729	1.779	1.827	1.871	1.918	1.962	2.009	90.0
100.0	1.093	1.127	1.161	1.198	1.233	1.270	1.309	1.350	1.387	1.428	1.470	1.510	1.553	1.595	1.637	1.679	1.717	1.765	1.811	1.852	1.895	1.932	1.977	100.0
110.0	1.094	1.127	1.161	1.198	1.233	1.270	1.308	1.349	1.386	1.425	1.466	1.505	1.547	1.587	1.625	1.666	1.700	1.747	1.789	1.827	1.868	1.900	1.937	110.0
120.0	1.094	1.127	1.161	1.198	1.233	1.269	1.307	1.346	1.383	1.421	1.461	1.499	1.538	1.576	1.611	1.650	1.680	1.726	1.763	1.798	1.837	1.866	1.897	120.0
140.0	1.093	1.126	1.159	1.196	1.230	1.266	1.303	1.340	1.372	1.408	1.443	1.477	1.511	1.546	1.574	1.608	1.624	1.665	1.703	1.732	1.768	1.785	1.804	140.0
160.0	1.092	1.124	1.157	1.192	1.224	1.259	1.295	1.330	1.355	1.388	1.416	1.446	1.473	1.500	1.522	1.540	1.560	1.587	1.627	1.656	1.705	1.718	1.725	160.0
180.0	1.092	1.122	1.153	1.187	1.216	1.248	1.284	1.317	1.334	1.361	1.381	1.409	1.427	1.449	1.462	1.480	1.500	1.520	1.540	1.575				180.0
200.0	1.089	1.119	1.148	1.180	1.206	1.236	1.246	1.298	1.308	1.328	1.353	1.373	1.390											200.0
240.0	1.085	1.113	1.137	1.161	1.182	1.191	1.218	1.245																240.0
280.0	1.080	1.106	1.125	1.141	1.156																			280.0
320.0	1.075	1.098																						320.0

Lengths of glass tubing approximately 25 cm. in length and 15 mm. inside diameter were used and on each a distance of 14 cm. was accurately marked off by file marks, 2 or 3 cm. being left at one end. This end was corked, the tube filled with liquefied honey and set up in an accurately vertical position. A thermometer graduated in $\frac{1}{10}°$ C. was clamped with its bulb accurately in the centre of the tube and about one inch below the surface of the honey. When the temperature of the honey had become reasonably constant, a steel ball-bearing ("S.K.F." $\frac{3}{16}$ in.) was dropped down the centre of the tube, its time of fall between the two marks being noted by means of a stopwatch. In the case of thin honeys the temperature was noted and the thermometer removed immediately before dropping the ball. In the case of thick honeys the ball was dropped down between the thermometer and the wall, and was then brought to the centre by tilting the tube before the ball reached the top mark. In these cases the temperature was noted when the ball passed the top and the bottom mark and the average temperature recorded. These precautions and the use of a sensitive thermometer are necessary since in general the accuracy of the method depends more upon the exactness of the temperature reading than upon anything else. Each reading was multiplied by its proper correction factor and the readings repeated until reasonably accurate checks for the viscosity at 25° C. were obtained.

The narrow tube is suitable for use where only small samples (3 oz. is quite sufficient) are available, and where liquefaction and subsequent cooling must be carried out fairly rapidly. Otherwise it would probably be more convenient to fill large graduated cylinders with the honey, allow them to come to constant temperature in a suitable room overnight, observing the time of fall in the morning. The need for carefully placing the tube in a strictly perpendicular position would thus be avoided, but on the other hand, following variations in room temperature, serious temperature gradients might develop between the different strata of the honey.

For the determinations of refractive indices a Zeiss industrial refractometer was used and found to be very convenient and satisfactory. The frequency with which results could be checked to closer than one in the fourth place justified estimating the readings to the fifth place and this was accordingly done. Readings were taken at room temperature and corrected to 25° C. on the basis of −.00023 per degree. The instrument was later checked against a Pulfrich and was found to give readings on the average .0003 too high. The observed readings were therefore corrected by this amount.

The determination of the moisture content was carried out by drying on sand in a vacuum oven according to the standard A.O.A.C. procedure. The dishes were in almost every case placed in the vacuum oven at 5.00 p.m. and removed for their first weighing next morning. They were then replaced and weighed for the second time early in the afternoon, when they generally showed a slight increase in weight, but not sufficient to invalidate the earlier reading. The work was done during the dry winter months, phosphorus pentoxide desiccators being used. Each determination was done in duplicate and the

results averaged. It is to be noted that in this particular case, the A.O.A.C. method cannot lead to any very great degree of accuracy since it directs that an amount of material shall be taken that will yield approximately 1 gm. of dry matter, and also that the heating shall be continued and weighings made every two hours until the difference between successive weighings is not more than 2 mg.

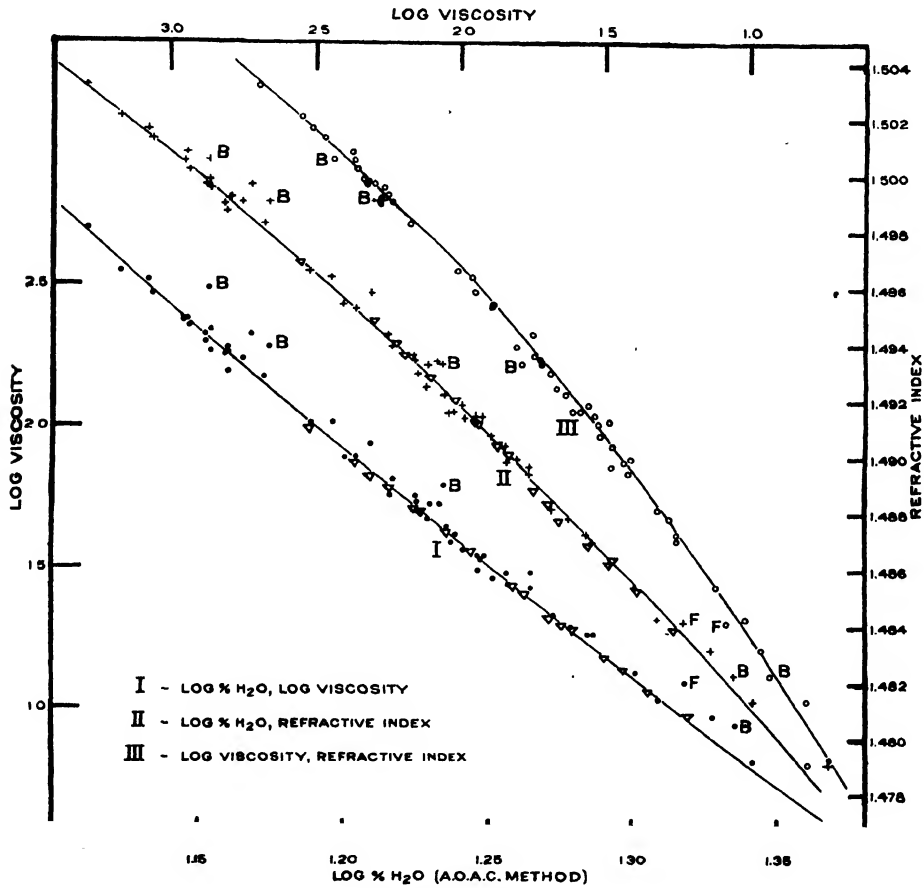

FIG. 2. *Results of viscosity, refractive index and A.O.A.C. moisture determinations, plotted to show the comparative accuracy of the results. The three points in the extreme lower right hand corner although not marked by the letter B were obtained from a buckwheat honey. Such honeys regularly give abnormal results.*

(*b*) *Results.* The results are shown in Fig. 2. In order to obtain lines which should be as straight as possible the co-ordinates chosen were: Curve I, log moisture-log viscosity; curve II, log moisture-refractive index and curve III, log viscosity-refractive index. The points which represent buckwheat honeys are specially designated by the letter *B*. These honeys show abnormally high viscosities and slightly less high refractive indices, judged by their moisture contents. The points designated *F* were obtained from a

TABLE III

DETERMINATION OF MOISTURE CONTENT FROM VISCOSITY AND REFRACTIVE INDEX* MEASUREMENTS

% H_2O	Time of fall through 14 cm., large ball, sec.	Refractive index at 25° C.	% H_2O	Time of fall through 14 cm., large ball, sec.	Refractive index at 25° C.	% H_2O	Time of fall through 14 cm. at 25° C., sec. Large ball	Time of fall through 14 cm. at 25° C., sec. Small ball	Refractive index at 25° C.	% H_2O	Time of fall through 14 cm. at 25° C., sec. Large ball	Time of fall through 14 cm. at 25° C., sec. Small ball	Refractive index at 25° C.
12.0	982	1.50550	14.6	164.8	1.49884	17.2	41.2		1.49234	19.9	12.65	30.3	1.48570
.1	906	529	.7	155.2	859	.3	39.1		210	20.0	12.16	29.1	548
.2	840	504	.8	145.9	832	.4	37.1		185	.1	11.70	28.0	526
.3	776	481	.9	137 7	807	.5	35.5		160	.2	11.25	26.9	503
4	719	454	15.0	129.8	781	.6	33.7		134	.3	10.84	25.9	481
.5	668	427	.1	122.2	756	.7	32.2		108	.4	10.45	25.0	460
.6	618	401	.2	116.4	735	.8	30.8		083	.5	10.07	24.1	437
.7	575	374	.3	109.4	706	.9	29.4		057	.6	9.68	23.2	416
.8	535	346	.4	102.8	681	18.0	28.1		032	.7	9.33	22.3	394
.9	496	319	.5	97.5	657	.1	26.9		009	.8	9.03	21.6	373
13.0	462	293	.6	92.1	632	.2	25.7		1.48983	.9	8.69	20.8	352
.1	428	266	.7	87.5	607	.3	24.7		959	21.0	8.38	20.1	329
.2	400	240	.8	83.2	581	.4	23.6		935	.1	8.07	19.3	305
.3	374	219	.9	79.1	557	.5	22 6		909	.2	7.82	18.7	284
.4	348	189	16.0	75.0	533	.6	21.7		885	3	7 53	18.02	261
.5	325	162	.1	70.8	507	.7	20.7		860	.4	7.28	17.42	240
.6	304	136	.2	67.4	482	.8	19.86	47.5	840	5	7 03	16.82	217
.7	285	111	.3	63.6	457	.9	19.05	45 6	811	6	6.76	16.18	195
.8	266	084	.4	60.7	431	19.0	18.28	43 7	786	.7	6 50	15.55	172
.9	251	061	.5	57.8	405	.1	17.54	42.0	762	.8	6.28	15.03	149
14.0	237.7	035	.6	54.8	382	.2	16.79	40.2	732	.9	6.10	14 60	127
.1	221.3	010	.7	52.1	357	.3	16.07	38.5	713	22 0	5.89	14 09	103
.2	208.4	1 49985	.8	50.0	333	.4	15.45	37.0	690	.1	5.72	13.69	080
.3	196.3	960	9	47.5	307	.5	14.83	35 5	666	.2	5 50	13.16	056
.4	185 4	940	17.0	45.4	283	.6	14 22	34.0	641	.3	5.31	12.70	031
5	174.2	910	.1	43.3	259	.7	13.65	32.7	618	.4	5.13	12.28	008
						.8	13.15	31.5	593	.5	4.95	11.84	1.47982

*Correction of refractive index = −0.00023 per °C.

sample of fermented honey. At first it was thought that all samples which had undergone even slight fermentation gave abnormal results, but this proved not to be the case. Just what effect serious fermentation has must be left an open question at present.

Superimposed upon Curves I and II are a number of points represented by triangles; these were not obtained from different honeys but by the dilution and concentration of only two samples. Since these points lie accurately upon the curves it is evident that the viscosity and refractive index of honeys which have been diluted and of those which are naturally thin do not differ appreciably.

Discussion

Explanation and Discussion of Tables III and IV

Curves I and II, Fig. 2, were used as the basis for Table III showing the relation between moisture content on the one hand and refractive index and viscosity on the other. In this table, the arbitrary values of viscosity which are used throughout this work are given in terms of the time of fall of a "large" and a "small" ball. These terms refer to steel ball-bearings $\frac{3}{16}$ and $\frac{3}{32}$ in. in diameter, respectively. The small ball should be used whenever possible in the testing of thin honeys since the experimental error in determining the longer time of fall is relatively less. The large balls actually used in this investigation were "S.K.F." ball-bearings weighing 0.4370 ± 0.0002 gm. A same sized "Hoffmann" ball-bearing weighed 0.4394 gm. It is probable, therefore, that the make of the steel ball is of no importance except in the most accurate work.

It will be seen that the "goodness of fit" is about the same for all methods, indicating that the limits of error characteristic of the individual determinations by each method are approximately the same.

Using Table III the actual moisture contents for the 60 honeys were calculated from the viscosity and refractive index figures which had been obtained and the results are shown in Table IV. The first group of honeys was supplied by the Bee Division, Central Experimental Farm, Ottawa, and consisted of a random selection from a collection of 211 honeys of the 1929 crop. Each sample had been analyzed by the Division of Chemistry (5, pp. 112-115) for moisture, ash, hydrogen ion concentration, titrateable acidity, nitrogen, invert sugar, sucrose, levulose, and dextrose. However no correlation could be discovered between these figures and the results of the present work.

The above samples were small ones, which during the course of time had lost several per cent of moisture and were thus of abnormally high viscosity. An effort was therefore made to obtain some honeys of higher moisture content, but none of very high moisture content could be found since the honey flow of 1931 had ceased before the lack of such honeys for investigation purposes was realized. The second group, however, represents moderately thin honeys supplied partly by the Dominion Apiarist and partly by the Ontario Honey Producers Co-operative, Ltd. The third group also consists of honeys of fairly high moisture content, being those most kindly sent by Mr. H. F. Wilson, Dept. of Economic Entomology, University of Wisconsin.

In the case of six honeys for which the results appeared to be abnormal and

TABLE IV
MOISTURE DETERMINATIONS ON SIXTY HONEYS

No. of sample	A	B	C	A-B	A-C	B-C
	% water by A.O.A.C. method	% water from ref. index	% water from viscosity			
Samples from Division of Bee Culture, Dominion Experimental Farm						
6	14.25	14.18	14.13	.07	.12	.05
10	13.61	13.60	13.67	.01	−.06	−.07
10a	13.96	13.91	14.02	.05	−.06	−.11
18	13.27	13.31	13.41	−.04	−.14	−.10
19	14.00	13.80	14.03	20	−.03	−.23
24	14.26	14.29	14.41	−.03	−.15	−.12
32	14.02	14.04	14.08	−.02	−.06	−.04
39	14.63	14.50	14 54	.13	.09	−.04
67	15.44	15.47	15 42	.03	−.02	−.05
69	15.70	15.56	15.42	.14	.28	.14
79	14 74	14.26	14 21	.48	.53	.05
113B	14.95	14 50	14 38	.45	.57	.12
117	14.19	14 25	14 30	−.06	−.11	−.05
120	16.15	15.75	15.65	.40	.50	.10
121	18 33	18 21	18 12	.12	.21	.09
124	14 46	14.47	14.45	− 01	.01	.02
130	15 85	15 93	15.95	−.08	−.10	−.02
142	16 78	16.67	16 67	−.11	−.11	.00
143	16 93	17 04	16 96	−.11	−.03	.08
152	12.93	12 89	12.91	.04	.02	−.02
154	14 47	14 45	14 38	02	.09	.07
158	14.88	14.80	14.77	.08	.11	.03
176	13.57	13.47	13.54	.10	.03	.07
183	14.42	14 42	14 47	.00	−.05	−.05
185	14 46	14 39	14 55	07	−.09	−.16
186B	23 35	22.80	21 73	.55	1.62	1.07
194B	14 24	13 90	13 61	34	.63	.29
202	14.20	14.22	14.16	− 02	04	− 06
Miscellaneous samples of higher moisture content						
1	18 19	18.16	18 20	03	−.01	−.04
2	17.61	17.53	17 57	08	04	− 04
3	17.45	17.56	17 42	− 11	.03	−.04
4	18.70	18 88	18.69	−.18	.01	−.19
5	17 30	17 47	17.23	−.17	.07	.24
6	17.24	17.48	17.35	−.24	−.11	.13
7	17.70	17 54	17.58	.16	.12	−.04
8	16.42	16.36	16.56	.06	−.14	−.20
9	16.77	16.66	16 59	.11	.18	.07
10	17.17	17.23	17.12	−.05	.05	.10
11B	17.15	16 79	16.39	.36	.76	.40
12B	21.62	21 45	21.01	.17	.61	.44
13	18.88	18.99	18.92	−.11	−.04	.07
14F	20.77	20.58	20.05	.19	.72	.53
15	21.22	21.04	20.81	.18	.41	.23
16	20.35	20.52	20.44	−.17	−.09	.08
17	18.36	18.24	17.87	.12	.49	.37
18	18.04	18.18	18.10	−.14	−.06	.08

TABLE IV—*Continued*

No. of sample	A: % water by A.O.A.C. method	B: % water from ref. index	C: % water from viscosity	A-B	A-C	B-C
American samples of high moisture content						
I	19.31	19.33	19.08	−.02	.23	.25
II	17.65	17 64	17.64	.01	.01	.00
III	17.42	17 34	17.49	.08	−.07	.15
IV	17.83	17.80	17.97	.03	−.14	−.17
V	16.47	16.52	16.31	−.05	.16	.21
VI	17 08	16 74	16 69	34	39	.05
VII	18.02	17.95	17.89	.07	.13	.06
VIII	19.96	20.01	19.84	−.05	.12	.17
IX	17.61	17.62	17.84	−.01	−.23	−.22
X	19 22	19.20	19.04	02	.18	.16
XI	16.82	16.91	16.86	−.09	−.04	.05
XII	16.95	16.80	16.71	15	.24	.09
XIII	16 00	15.99	15.97	01	.03	.02
XIV	17.61	17.62	17.84	−.01	−.23	−.22

TABLE V

COMPARISON OF RESULTS OF AUERBACH AND BORRIES WITH THOSE OBTAINED BY USE OF TABLE IV

Ref. index at 40° C. (A. and B.)	Ref. index corrected to 25° C.	% H_2O calculated by A. and B.	% H_2O calculated from Table IV	Difference
Normal honeys				
1.4938	1.4972	15.36	15.25	−.11
1.4906	1.4940	16 61	16.52	−.09
1.4904	1.4938	16 69	16.60	− 09
1.4917	1.4951	16 18	16.10	−.08
1.4882	1 4916	17.55	17.50	−.05
1.4906	1 4940	16 61	16 52	− 09
1.4863	1 4897	18 29	18 25	−.04
1.4855	1.4889	18.60	18.58	−.02
1.4829	1.4863	19.62	19.65	+.03
1.4811	1.4845	20 32	20.44	+.12
15-Year-old honeys				
1.4957	1.4991	14.62	14.50	−.12
1.4906	1.4940	16.61	16.52	−.11
1.4904	1.4938	16.69	16.61	−.08
1.4865	1.4899	18.21	18.17	−.04
1.4857	1.4891	18.52	18.50	−.02
1.4878	1.4912	17.70	17.65	−.05
1.4864	1.4898	18.25	18.21	−.04
Special honeys				
1.5017	1 5051	12.27	12.12	−.15
1.4984	1.5018	13.56	13.44	−.12
1.4882	1 4916	17.55	17.50	−.05
1.4969	1.5003	14.15	14.02	−.13
1.4994	1.5028	13.17	13.05	−.12
1.4741	1.4775	23.05		

therefore open to suspicion, the determinations were repeated, but, except in the case of two, the resulting corrections were negligible. It may safely be concluded, therefore, that the discrepancies in the recorded results are due, not so much to experimental error in technique, as to the theoretical limits of accuracy of the different methods.

Comparison with the Work of Auerbach and Borries

It is satisfactory to note the almost theoretical agreement between the refractive index results arrived at in this work and those obtained by Auerbach and Borries (1). These workers determined (a) the density to five places of decimals (using diluted samples and a pycnometer), (b) the refractive index at 40° C., and (c) the moisture content by a special drying method, of 10 honeys. From these results they deduced empirical equations for the relationships between refractive index, density and moisture. Using these equations they calculated, on the basis of both refractometric and density measurements, the moisture content of 23 honeys, seven of which they classified as 15-year-old honeys and six as "special honeys". Since in their paper they also published the actual refractive indices at 40° C. from which they made their calculations, it has been possible to check their calculations against those developed in the present investigation. A comparison of results is shown in Table V.

Hydrometer Method to be Investigated

Further, the work of Auerbach and Borries brings to the fore the question of the accuracy and suitability of the hydrometer. As will be seen from the last column, Table VI, the differences they obtained between moisture determined by density and moisture determined by refractive index are very small. True, the agreement between these and their absolute figures is not as good, but the considerably better agreement obtained between refractive index and absolute moisture determinations in the present work seems to show that the source of their discrepancies must lie at least partly in the moisture determinations themselves and not altogether in the indirect methods. However, their figures do show that density in itself is as accurate an indication of moisture content as is refractive index.

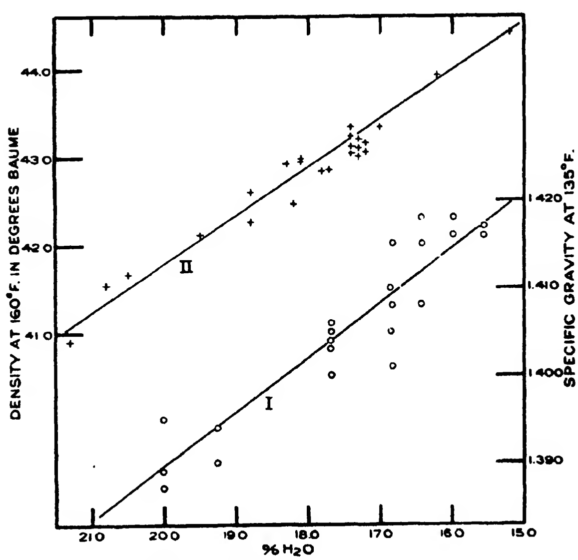

FIG. 3. *Curves indicating the degree of accuracy at present obtainable by hydrometer methods on undiluted honeys.*

On the other hand the determination of the density of a diluted sample of

TABLE VI

AUERBACH AND BORRIES' FIGURES FOR THE COMPARISON OF DIRECT AND INDIRECT METHODS OF MOISTURE DETERMINATION

D % water by direct weighing	E % water from density	F % water from ref. index	D-E	D-F	E-F
Normal honeys					
15.71	15.49	15.36	0.22	0.35	0.13
15.80	16.43	16.61	−0.63	−0.81	−0.18
16.27	16.63	16.69	−0.36	−0.42	−0.06
16.46	16.13	16.18	0.33	0.28	−0.05
16.99	17.54	17.55	−0.55	−0.56	−0.01
17.27	16.59	16.61	0.68	0.66	−0.02
17.34	18.18	18.29	−0.84	−0.95	−0.11
19.11	18.66	18.60	0.45	0.51	0.06
19.83	19.48	19.62	0.35	0.21	−0.14
20.66	20.43	20.32	0.23	0.34	0.11
15-Year-old honeys					
15.03	14.76	14.62	0.27	0.41	0.14
16.37	16.72	16.61	−0.35	−0.24	0.11
16.95	16.85	16.69	0.10	0.26	0.16
17.63	18.24	18.21	−0.61	−0.58	0.03
17.75	18.57	18.52	−0.82	−0.77	0.05
18.15	17.93	17.70	0.22	0.45	0.23
18.53	18.39	18.25	0.14	0.28	0.14
Special honeys					
13.39	12 43	12.27	0.96	1.12	0.16
14.66	13.35	13.56	1.31	1.10	−0.21
17.94	17.60	17.55	0.34	0.39	0.05
15.67	14.35	14.15	1.32	1.52	0.20
13.03	13.10	13.17	−0.07	−0.14	−0.07
23.52	23.06	23.05	0.46	0.47	0.01

honey in a pycnometer bottle and the determination of the density of a heated undiluted honey sample using a hydrometer are two entirely different things. The first is accurate but is such a slow and delicate procedure as to be useless in practical work. The second method is simple and fairly rapid but the actual results obtained may be exceedingly poor. Thus, using a special honey hydrometer supplied by the Dominion Apiarist, and which was designed for testing small samples of honey, the results shown in curve I, Fig. 3 were obtained. As will be seen, the individual density determinations on one and the same sample vary by an amount corresponding to over 2% of moisture. For comparison, some results supplied by Mr. L. Skazin, of these Laboratories, obtained with the use of a larger and more sensitive instrument belonging to the Ontario Honey Producers Co-operative, Ltd., are shown in curve II, Fig. 3. As will be seen, these are decidedly better. However one of the drawbacks is that very much larger samples are required for testing purposes.

It will be seen that the whole question of the practical value of the hydro-

meter method depends upon whether or not it is possible to devise an instrument which will be accurate as well as convenient. Work will be undertaken along these lines.

In the meantime, one or two remarks may be made with reference to the question as to which of the two methods, viscosity or refractive index, is the more suited to practical work. Where the cost of the instrument is a matter of minor consideration, it is probable that the refractive index method is the more suitable because of the ease and rapidity with which readings can be made. However, great care should be taken to secure a representative sample and to take the readings with the utmost precision. On the other hand, the apparatus for the measurement of viscosity is much cheaper, a good thermometer and a stopwatch being practically all that is required. Further, the measurements may be carried out by a less skilled observer, especially if large samples sufficient to fill reasonably wide tubes are available.

Summary

(1) A new method for the determination of moisture in honey, one dependent on viscosity, has been studied and found practicable.

(2) Viscosity, refractive index, and absolute moisture determinations have been carried out on 60 honeys.

(3) Based upon the above figures, tables have been drawn up which make it possible to determine moisture content simply and rapidly, either from viscosity or from refractive index measurements, with an error in all probability no greater than that of the standard A.O.A.C. method. However, buckwheat honeys and possibly those which have become fermented, give abnormal results.

(4) The ground has been cleared by preliminary work for a study of the degree of accuracy obtainable with carefully designed honey hydrometers.

Acknowledgments

The author wishes to acknowledge the help and advice received from many quarters: From colleagues at the Research Laboratories; from the Dominion Apiarist, Mr. C. B. Gooderham, who supplied the majority of the samples, and whose advice and criticism made clear many of the practical aspects of the problem; from Mr. W. A. Weir and Mr. M. Pettit of the Ontario Honey Producers Co-operative, Ltd., whose interest has shown itself in most practical form, and at whose plant routine tests have been carried out; from the Division of Chemistry, Central Experimental Farm, which supplied original data on the large collection of honeys analyzed there; and finally from Mr. H. F. Wilson of the Department of Economic Entomology, University of Wisconsin, who very kindly supplied the American honey samples, and whose correspondence has been most helpful and encouraging.

Bibliography

1. Auerbach, F. and Borries, G. Z. Nahr. Genussm. 48: 272-277. 1924.
2. Lochhead, A. G. and McMaster, N. B. Sci. Agr. 11: 351-360. 1931.
3. Marvin, G. E. and Wilson, H. F. J. Econ. Entomol. 24: 603-604. 1931.
4. Schneller, M. J. Assn. Off. Agr. Chem. 9: 156-165. 1926.
5. Shutt, Frank T. Rep. of the Dom. Chem., Dept. of Agric. March. 1930.

THE EFFECT OF COOKED POTATO IN CONJUNCTION WITH FERMENTABLE CARBOHYDRATE IN BREADMAKING[1]

By R. H. Harris[2]

Abstract

A series of 11 flours of different types and baking strengths were baked by a variety of methods in order to determine the effect of adding fermentable sugar to the dough in conjunction with cooked white and sweet potato. Preliminary bakings using a simple formula of flour, water, yeast, and salt, with and without sucrose, indicated the value of sucrose in flours of low diastatic power.

The addition of cooked white potato in the absence of sucrose gave still poorer results with these low diastatic flours, but improved the better flours. The addition of sucrose increased the response of all flours, the maximum stimulation being obtained when both malt and sucrose were added. Evidently, cooked white potato is not a satisfactory substitute for fermentable sugars.

It was found that cooked sweet potato not only supported yeast activity, but also stimulated it. However, it also imparted a deleterious dark tint to the loaf, thus lowering the color score.

The addition of potassium bromate without sucrose resulted in a greater loaf volume than that obtained by the use of the simple formula with sucrose, except in the case of two flours, particularly low in diastatic activity. The inclusion of diastatic malt increased the loaf volumes of these two flours.

No significant relationship was found between protein content and loaf volume, in the absence of added fermentable carbohydrate. The addition of sucrose and malt to bakings with potato extract resulted in correlations equal to those obtained with other flour improvers.

Introduction

While the standard baking procedures in use at present require a certain amount of fermentable sugar as one of the chief ingredients, formulas which did not include this item have been used to some extent. Schnelle (12) employed a formula without sugar when studying different varieties of German wheat. He used a procedure including 4% yeast, 2% salt and water in proportion to the absorption of the flour. The fermentation temperature was 35°C., the dough being punched after 30 min. and the loaves panned after an additional 30 min. The proofing time was not rigidly fixed, and upon occasion varied as much as 30 min. or more. This method has been criticized by Jorgenson (8) on the grounds that no adequate distinction is made between the gassing power of the dough and the baking strength proper, due to the strong probability of yeast starvation resulting from lack of fermentable sugars necessary for the support of yeast activity during the fermentation period.

To date very little additional data have been published regarding the use of a baking formula without sugar, although various research workers have doubtless employed such a method at different times. It is quite probable, however, that some of the results obtained by this system of baking will be published in the near future.

As pointed out by Jorgenson, the principal weakness of a baking formula without fermentable sugar is the danger of yeast starvation in flours of low

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diastatic activity, which, with the addition of sufficient sugar or malt to support yeast activity, would have produced satisfactory loaves. The lack of fermentable sugar confuses the issue, and would condemn a flour on the grounds of low diastatic activity and lack of baking strength. Further, commercial bakers almost universally use diastatic malt as well as sugar in some form in their formulas. A flour condemned in the baking laboratory because of failure to stand up without sugar could very possibly produce an excellent loaf when malt and sugar were added to supplement the available flour sugars.

Harris (5) published the results of a study on the baking qualities of a series of flours of various types with formulas which included different quantities of cooked potato. These formulas contained 2.5% of sugar in every case in addition to the potato material, and a marked improvement was noted in loaf volume and baking score for practically every flour examined. In view of the discussion concerning the omission of sugar, the author deemed it advisable to investigate further the use of cooked potato as applied to baking, especially with regard to its use in a formula without added fermentable sugar. Housewives are generally accustomed to the use of cooked potato in their home baking and would probably be interested in the production of equally satisfactory bread without the use of sugar. A further source of interest was the possibility of explaining part of the beneficial properties of the potato on the hypothesis that the carbohydrate from the potato serves as food for the yeast during the fermentation period.

TABLE I

DESCRIPTION AND CRUDE PROTEIN CONTENT OF FLOURS

No. of flour	Description	Crude protein, %
31	Soft winter wheat pastry	7.5
32	Unbleached middling's flour, 1930 and 1931 crops	10.7
33	First patent, blend of 1930 and 1931 crops	11.7
34	Baker's patent, milled from weathered 1931 crop	11.7
35	Baker's patent, milled from 1930 wheat	12 5
36	Second patent, from blend, 1930 and 1931	13.1
37	Unbleached straight, experimental 1° Reward	13.1
38	Baker's patent, from 1930 crop	13.5
39	Unbleached experimental straight No. 6 frosted	13.8
40	Unbleached break flour, blend 1930 and 1931	16.1
41	Strong first clear	17.6

A plan tentatively adopted was to bake a series of representative flours of varied baking strengths and characteristics, using formulas including (a) sugar, (b) no sugar, (c) potato and sugar, and (d) potato and no sugar.

Materials and Methods

The baking procedure was as follows: the doughs were mixed by hand in earthenware bowls and run in pairs at intervals of five minutes, the fermentation, proofing time and temperatures being those of the Blish (1) standard method. The following formula was used: flour, 100 gm.; yeast, 5 gm.; sugar, 2.5 gm.; salt, 2 gm.; distilled water as required for proper dough consistency.

This is called the simple formula No. 1 in this paper. The simple formula No. 2 is identical except for the omission of the sugar, while the potato treatments were obtained by adding to these two simple formulas 40 cc. of cooked potato extract at a suitable temperature. Potato extract rather than mashed cooked potato was used in the present instance because it was found to be more easily incorporated in the dough. In the subsequent bakings carried out with malt, potassium bromate, and cooked sweet potato, the two simple formulas were used in the same manner with the addition of the ingredients mentioned. The sweet potato was weighed directly into the 100 gm. of flour before mixing and the potassium bromate was introduced by means of a pipette, 2 cc. of the solution containing 0.002 gm. of the improver. The diastatic malt was weighed as accurately as possible and added to the flour before the other ingredients. Owing to the large quantities of other liquids necessary in some cases, it was more convenient to weigh the malt directly rather than to incorporate it as an aqueous solution.

A baking score was computed for these flours as follows:

Loaf volume	$\times$ 0.1	
Color of loaf	$\times$ 1.0	Maximum value 20
Grain of loaf	$\times$ 1.0	Maximum value 10
Texture of loaf	$\times$ 1.0	Maximum value 10

The sum of these individual scores was considered to be the baking value of the flour. Symmetry of the loaf was not scored, owing to its high correlation with loaf volume, and the tendency to cause very high total scores when used with strong protein flours.

The potato extract was prepared by boiling peeled and sliced white potatoes for approximately 35 min., the vessel being kept covered during the cooking process to avoid loss of moisture as much as possible. The thick liquid was then drained from the potato and allowed to stand and cool. After cooling to 20°C., the supernatant liquid was decanted from a white flocculent precipitate, and kept in an ice box until required. It was found to be impossible to keep the thick material in suspension when the liquid had cooled, with resulting variations in the concentration of potato. It appeared also, that a more uniform product was obtained by chilling and then draining off the liquid. This extract was kept not more than three or four days before using.

The sweet potato was prepared in a very similar manner, except that the water was allowed to evaporate during the cooking. The soft, moist slices were then thoroughly broken up and completely mixed by stirring. The potato, of a dark brown color, was partially dried and cooled to room temperature before using.

The series of flours used in this study was selected with a view to obtaining representative flours of the various types used in experimental baking.

Results

With simple formulas. In Table II are shown the loaf volumes and baking scores obtained with these flours when baked by the simple formulas. The

TABLE II
COMPARATIVE BAKING DATA OBTAINED WITH SIMPLE FORMULAS NO. 1 AND NO. 2

	Simple formula No. 1 (2½% sugar)					Simple formula No. 2 (no sugar)				
No.	Loaf volume, cc.	Color	Grain	Texture	Score	Loaf volume, cc.	Color	Grain	Texture	Score
31	330	12.0	4	2.0	51	290	10.0	4.5	1.0	44
32	550	12.0	9	8.5	84	515	13.0	9	8.5	82
33	520	20.5	9	9.0	90	530	19.5	5	5.0	82
34	515	17.0	6	6.0	80	560	19.0	3	4.0	82
35	514	16.5	8	7.5	83	530	16.0	7	7.0	83
36	540	16.5	8	8.0	86	560	17.0	6	6.0	85
37	540	15.0	8	7.0	84	465	16.0	7	6.0	65
38	525	16.0	6	7.0	81	380	15.0	3	3.0	59
39	545	11.5	7	7.0	80	475	11.0	5	6.0	69
40	570	12.0	7	7.0	83	460	11.5	7	6.0	70
41	540	8.0	4	3.0	69	570	6.0	2	1.0	66

loaf volumes exhibit no decided increase with increasing protein. In several samples, namely, Nos. 37, 38, 39 and 40, very decided decreases in loaf volume were evident when no sugar was used in the formula, as compared with the corresponding values obtained when sugar was included. This was probably due to starvation of the yeast, as pointed out by Jorgenson. No great differences in loaf color are displayed by the two systems of baking, but the grain and texture scores appear rather lower in the series baked without added sugar. With two exceptions, the latter series has a decidedly lower baking score as compared with the former. This is due primarily to lower volume, or lower grain and texture scores.

TABLE III
COMPARATIVE BAKING DATA OBTAINED BY ADDING 40 CC. OF POTATO EXTRACT TO SIMPLE FORMULAS NO. 1 AND NO. 2

No.	Simple formula No. 2 plus extract					Simple formula No. 1 plus extract				
	Loaf volume, cc.	Color	Grain	Texture	Score	Loaf volume, c.c.	Color	Grain	Texture	Score
31	260	10.0	4.0	2	42	290	10.5	4.0	2.0	45
32	620	13.5	9.5	8	93	610	14.0	8.0	7.0	90
33	560	22.0	8.5	9	95	630	22.5	9.0	9.5	104
34	750	21.0	4.0	5	105	692	21.5	5.0	4.0	99
35	550	16.0	6.5	7	84	628	17.5	7.0	6.5	94
36	600	18.0	7.5	8	93	650	19.0	7.0	8.5	99
37	405	14.0	5.0	5	64	560	15.5	4.0	4.0	79
38	340	13.0	3.0	2	52	550	16.0	6.0	7.0	84
39	405	10.5	5.0	5	61	645	13.0	5.5	6.5	89
40	435	10.0	3.0	4	60	680	12.0	6.0	6.0	92
41	530	7.0	2.0	1	63	640	6.5	2.5	2.0	75

With potato extract. In Table III are shown the loaf volumes and scores obtained by baking the flours with 40 cc. of potato extract added to the simple formulas and in Table IV those obtained with the addition of both 2% diastatic malt and potato to the No. 1 formula. The malt was used to increase the available supply of fermentable sugars when the yeast was stimulated by the potato material.

TABLE IV

BAKING DATA OBTAINED BY ADDING 40 CC. OF POTATO EXTRACT AND 2% DIASTATIC MALT TO SIMPLE FORMULA NO. 1

No.	Loaf volume, cc.	Color	Grain	Texture	Score	No.	Loaf volume, cc.	Color	Grain	Texture	Score
31	385	11.0	3.0	1.0	53	37	660	16.0	6.5	5.0	93
32	540	12.5	7.0	6.0	79	38	610	16.0	7.0	6.0	90
33	700	21.0	6.0	6.0	103	39	730	13 0	6.0	5.5	97
34	670	18.5	8.0	7.0	100	40	930	12.0	5 0	5.0	115
35	700	19.0	5.0	5.0	99	41	790	6.0	2.0	2.0	89
36	750	18.0	5.0	5.0	103						

The use of potato in addition to simple formulas Nos. 1 and 2 seems to accentuate the differences in loaf volume yielded by these formulas in the absence of potato. There appears to be a tendency toward higher color score when sugar is included in the potato bakings. These two factors, loaf volume and color score, tend to raise the baking score when sugar is used. The loaf color appears to fall off slightly when malt is used, due no doubt to the dark tinge contributed by this ingredient.

With sweet potato. Geddes and Winkler (4) found that honey and sucrose were equally valuable for the support of yeast fermentation in flour doughs. With their findings in mind and in view of the negative results obtained with cooked white potato, it was decided to try cooked sweet potato as a flour improver in a baking without added sugar. Flour No. 40, being exceedingly strong, and apparently lacking in diastatic power, was selected as a suitable sample to combine with various sweet potato treatments. The data obtained by combining this flour with increasing amounts of cooked sweet potato without other added fermentable sugars are shown in Table V. It would appear

TABLE V

THE EFFECT OF ADDING VARIOUS PERCENTAGES OF COOKED SWEET POTATO TO SIMPLE FORMULA NO. 2

Concentration of potato, %	Loaf volume, cc.	Color	Grain	Texture	Score	Remarks
0	460	11.5	7	6	70	Brown tinge imparted to the loaf by the potato
5	470	10.0	7	6	70	
10	520	9.0	5	5	71	
20	690	8.0	3	3	83	
30	750	7.5	3	4	89	

from the data presented in this table that the sweet potato not only acts as a substitute for sugar but also has a decidedly stimulating effect upon the baking properties of the dough. The color score is lowered, however, by the presence of the brown potato material.

To obviate this difficulty, an attempt was made to obtain an extract which would not have such a marked effect upon the color score. Accordingly, extract No. 1 was prepared by boiling 180 gm. of sliced sweet potato in 360 cc. of water, the water being replenished to offset evaporation. After 45 min. the liquid was drained from the still intact pieces of potato, and the process repeated on the residue with a fresh portion of water, obtaining a second extract, No. 2. In this case, boiling was continued for only 20 min. The extracts were then cooled and used separately with simple formula No. 2.

An addition of 40 cc. of extract No. 1 gave a loaf volume approximately equal to the corresponding value obtained with formula No. 1 alone. The second extract was very weak in its effect, and reduced the loaf volume, probably due to a slight stimulation of the yeast without a corresponding increase in the supply of fermentable sugar.

A third extract, No. 3, was made by using 200 gm. of sweet potato and 200 cc. of water, employing the same procedure as before. This extract

TABLE VI

THE EFFECT OF ADDING VARIOUS EXTRACTS OF SWEET POTATO TO SIMPLE FORMULA No. 2

Volume of extract added, cc.	Loaf volume, cc.	Color	Grain	Texture	Score
Extract No. 1. 180 gm. of sweet potato boiled for 45 min. in 360 cc. of water					
10	450				
20	490				
30	510				
40	560				
Extract No. 2. Made by boiling the residue from No. 1 for 20 min. in 360 cc. of water					
30	420				
40	410				
Extract No. 3. 200 gm. of sweet potato boiled for 45 min. in 200 cc. of water					
10	475	10.0	7	6	70
20	500	10.5	5	5	70
30	610	12.0	5	5	83
40	650	14.0	4	4	87
Extract No. 3 plus 2½% of sugar					
10	610	14.5	7	7	89
20	640	12.0	7	7	90
30	650	11.5	7	7	90
40	670	12.0	6	7	92

was used in portions of 10, 20, 30 and 40 cc., without sugar and with 2½% of sugar. The loaf volumes and scores assigned the loaves made with these extracts are shown in Table VI. The higher concentrations of this extract increased the loaf color score over the values for the cooked sweet potato, but the loaf volume was not as high as for the 30% mashed potato. The baking score was very similar to that assigned to the loaves made with mashed potato, but was increased when sugar was added to the formula.

In view of the larger loaves obtained with the sweet potato itself as compared with the extract, as well as the large quantity of potato necessary to furnish sufficient liquid to treat the entire series of flours, dried cooked sweet potato was then used with all the flours in a concentration of 20%. The resulting loaf volumes and baking scores are shown in Table VII.

TABLE VII

BAKING DATA OBTAINED BY ADDING 20% SWEET POTATO TO SIMPLE FORMULA No. 2 (no sugar)

No.	Loaf volume, cc.	Color	Grain	Texture	Score	No.	Loaf volume, cc.	Color	Grain	Texture	Score
31	365	5.0	4.0	3.0	48	37	665	13.0	4.5	4.0	88
32	566	8.0	5.0	5.0	75	38	590	13.0	4.0	5.0	81
33	630	13.0	6.0	4.5	86	39	700	8.5	5.0	3.0	86
34	635	14.0	6.0	5.5	89	40	840	8.0	3.0	3.0	98
35	600	12.0	4.5	5.5	82	41	700	7.0	4.0	4.0	85
36	682	13.5	4.0	4.0	90						

An examination of this table shows that the loaf volumes tend to increase with increasing flour protein. The color scores are all rather low, owing to the darkening effect of the sweet potato, while grain and texture scores reflect the "opening up" of the interior of the loaf through the action of the potato. Those flours which, when baked without sugar gave poor loaf volumes as compared with the values yielded with sugar, gave satisfactory results in the presence of the sweet potato.

With potassium bromate and with potassium bromate plus malt. Two further bakings were made with this series of flours, using 0.002% potassium bromate with formula No. 2, and 0.002% potassium bromate plus 2% diastatic malt with formula No. 1. Various workers in the field have used flour improvers, including malt, with marked success in bringing out the full potentialities of a flour. MacLeod (11), Larmour and MacLeod (10), and Larmour (9), in a study of different series of Canadian wheat flours, found high correlations between crude protein and loaf volume when potassium bromate, bromate and malt, or Arkady and malt were included in the standard formula. Without these flour improvers, the correlations were significantly lower. Harris (6, 7) found also, in an investigation of the relations between total protein, protein peptizability, and loaf volume, that these improvers raised the correlations existing between the variables examined. Geddes (3), studying the effect of heat treatment upon flour, concluded that potassium bromate in the baking

formula assisted in detecting injury to the flour quality, which injury, if the standard or basic formula alone had been used, would have escaped observation.

TABLE VIII

BAKING DATA OBTAINED WITH SIMPLE FORMULA NO. 2 PLUS 0.002% OF POTASSIUM BROMATE, AND WITH SIMPLE FORMULA NO. 1 PLUS 0.002% OF POTASSIUM BROMATE PLUS 2% OF DIASTATIC MALT

	Simple formula No. 2 plus 0.002% of potassium bromate					Simple formula No. 1 plus 0.002% potassium bromate plus 2% malt				
No.	Loaf volume, cc.	Color	Grain	Texture	Score	Loaf volume, cc.	Color	Grain	Texture	Score
31	295	16.5	3.0	1.0	50	355	12.0	2.0	2.0	51
32	530	17.0	7.0	8.0	85	488	13.0	8.0	7.0	67
33	570	21.0	5.0	7.0	90	540	15.0	6.0	6.0	81
34	580	16.0	8.0	6.0	88	580	15.0	5.0	4.5	82
35	540	19.0	4.5	6.0	83	560	14.0	6.5	6.0	82
36	620	19.0	7.5	8.0	96	620	14.0	6.0	7.0	89
37	600	19.5	6.0	5.0	90	580	15.0	5.0	5.0	83
38	470	18.0	8.0	7.0	80	580	12.0	5.0	6.0	81
39	690	16.0	7.0	5 0	97	680	13.5	3.0	3.0	87
40	705	13 0	7.5	7.0	98	870	15.5	3.5	3.5	109
41	675	9.0	4.0	4.5	85	820	9.0	4.0	4.0	99

In view of the utility of potassium bromate in evaluating flours, it seemed advisable to investigate the action of this bromate upon the flours used in the present study, and to compare the resulting data with those obtained by the methods already discussed. The baking results from the two series when treated with potassium bromate, with and without added sugar, are shown in Table VIII. Several of the flours showed a surprising increase in loaf volume when sugar and malt were included in the formula together with potassium bromate. The color score was decidedly lower when malt was present, this tendency being, in some instances, reflected in the baking score. The stronger flours, when sugar and malt were used, tended to give lower grain and texture scores as compared with the corresponding values obtained with bromate alone.

Comparisons of loaf volumes obtained with the various baking formulas. The loaf volumes obtained in all the bakings, together with the flour protein, are summarized in Table IX. Glancing first at the values yielded by the two simple formulas it is seen that No. 1 shows evidence of an increase in loaf volume, with increasing protein. This trend is not evident in the data yielded by method No. 2, where a sharp fall in volume occurs in samples Nos. 37, 38, 39 and 40. These flours appeared to be lacking in diastatic activity and consequently were unable to support properly yeast activity during the fermentation period. This fact is still more noticeable when the loaf volumes yielded after the addition of 40 cc. of potato extract are considered. Further decreases in loaf volume are evident with these particular flours when the above extract is used without the addition of fermentable sugar, but when sugar is added,

TABLE IX

COMPARATIVE LOAF VOLUMES (cc.) OBTAINED BY THE VARIOUS FORMULAS, ON A BASIS OF 13.5% MOISTURE

No.	Protein, %	Simple formulas		Potato extract (40 cc.) plus simple formulas:			Sweet potato (20%) plus simple formula No. 2	KBrO₃ (0.002%) plus simple formulas:		Average loaf volume, cc.
		No. 1	No. 2	No. 1	No. 2	No. 1 + 2% malt		No. 2	No. 1 + malt (2%)	
31	7.5	330	290	290	260	385	365	295	355	321
32	10.7	550	515	610	620	540	566	530	488	552
33	11.7	520	530	630	560	700	630	570	540	585
34	11.7	515	540	692	750	670	635	580	580	620
35	12.5	514	530	628	550	700	600	540	560	578
36	13.1	540	560	650	600	750	682	620	620	628
37	13.1	540	465	560	405	660	665	600	580	559
38	13.5	525	380	525	340	610	590	470	580	502
39	13.8	545	475	645	405	730	700	690	680	609
40	16.1	570	460	680	435	930	840	705	870	686
41	17.6	540	570	640	530	790	700	675	820	658
Average		517	483	595	496	679	634	570	609	573

NOTE:—Simple formula No. 1 contains 3% yeast, 2% salt, 2½% sugar.
Simple formula No. 2 contains 3% yeast, 2% salt, no sugar.

large increases in loaf volume occur. These loaf volumes become still larger when diastatic malt, insuring a plentiful supply of fermentable sugar, is also present. The addition of potato in the absence of sugar gave loaf volumes in some cases appreciably greater than those obtained without potato but with sugar. In these samples a sufficient supply of fermentable sugar must either have existed from the beginning or must have been formed to meet the increased demands of the stimulated yeast fermentation. On the addition of both sugar and malt to the formula, not only did these flours show no further increase in loaf volume, but, in the cases of Nos. 32 and 34, there was a decrease. Flour No. 33 gave satisfactory results without added sugar, but produced a larger loaf when a supply of fermentable sugar was assured. The bakings with 40 cc. of potato extract plus sugar and malt generally yielded the largest loaves of the entire set of bakings represented in Table IX.

The treatments with 20% cooked sweet potato show increased loaf volumes in those flours which were unsuitable for use in a formula without sugar, and appear to give higher values than were yielded by the treatments with 40-cc. extracts of white potato plus 2½% sugar. When in addition diastatic malt was added, the use of extracts resulted in still greater volumes. These tendencies are reflected in the fact that the average loaf volumes are highest for the bakings with potato extract plus sugar and malt, and next highest for the sweet potato bakings. Sweet potato, in so far as loaf volume is concerned, is apparently able to supply any deficiency existing in a flour as well as, or better than, sucrose itself, but is not quite as satisfactory for this purpose as white potato, sucrose and diastatic malt.

The addition of .002% of potassium bromate resulted in greater loaf volume in both the presence and absence of 2½% of sugar, as is particularly exemplified by flours such as Nos. 37, 38, 39 and 40, the initial showing of which, owing to

the absence of sugar, was rather poor. Only two flours gave smaller loaf volumes when baked with potassium bromate and without sugar, than when baked with sugar and without potassium bromate. One of these flours was very weak, while the other, No. 38, acted throughout as if it were lacking in the ability to produce any appreciable amount of fermentable sugar.

When 2% of diastatic malt was added in addition to the potassium bromate, an appreciable increase in loaf volume resulted in the cases of higher protein flours. The averages obtained with bromate plus malt are the third highest in the table. The final average loaf volumes for all the bakings show the effect of the low diastatic flours which, yielding very low volumes for some of the bakings, lower the average results.

Upon examination of the data presented in Table IX with respect to the behavior of individual flours under the various treatments, it appears that No. 34, which was commercially milled from wheat which had been exposed to several rains interspersed with drying weather, gave exceptionally satisfactory results without the addition of any form of sugar. This tendency can probably be explained on the grounds of increased diastatic activity due to exposure to moisture and warm weather while in the stook. Sample No. 40 was quite different in its response to the various baking formulas. Though an exceedingly strong flour, it was unable to produce large loaves without added sugar, there being a difference of 495 cc. between the minimum and maximum loaf volumes. The two experimental flours, Nos. 37 and 39, exhibit the same tendency in lesser degree. Potassium bromate appears to function in the cases of these flours in much the same manner as a formula containing sugar in addition to a flour improver. No. 39 showed no trace of having suffered injury through frost damage. The clear flour, No. 41, did not, with any of the treatments, produce as large a loaf as did No. 40, although it contained more protein than the latter.

Discussion of correlation coefficients. The correlation coefficients computed between flour protein and loaf volume are given in Table X. The significance

TABLE X

CORRELATION COEFFICIENTS COMPUTED BETWEEN FLOUR PROTEIN AND LOAF VOLUME

Baking formula	Correlation coefficient
Simple No. 1	+.734
Simple No. 2	+.494
Simple No. 1 plus 40 cc. potato extract	+.620
Simple No. 2 plus 40 cc. potato extract	+.104
Simple No. 1 plus 40 cc. potato extract plus 2% malt	+.866
Simple No. 2 plus 20% sweet potato	+.856
Simple No. 2 plus 0.002% potassium bromate	+.838
Simple No. 1 plus 0.002% potassium bromate plus 2% malt	+.954
Average loaf volume, by all formulas	+.804
Value of r at 5% point	+.602
Value of r at 1% point	+.708

NOTE:—Formula No. 1 contained 3% yeast, 2% salt, 2½% sugar.
Formula No. 2 contained 3% yeast, 2% salt, but no sugar.

of these coefficients may be judged by comparison with the points of minimum significance according to the number of pairs of observations. These values have been tabulated by Fisher (2). The coefficients are calculated from 11 pairs of values and the 5% and 1% points are respectively .602 and .708.

The coefficients obtained by the use of the baking methods without added fermentable sugar are not significant, and show the importance of this ingredient in baking a series of flours of the types used in this study. When potato extract was used, the inclusion of sugar in the formula raised the correlation coefficient to a value between the 5% and 1% points. When diastatic malt was also added, the coefficient becomes significant for the 1% point.

In order to determine whether the differences between any of the constants were significant, the Z test proposed by Fisher (2, p. 170) was used. This test depends upon the substitution of a value, Z, for each value of the correlation constant *r*.

The significance of the difference between any two correlations may be expressed by the ratio of the difference in Z values ($Z_1 - Z_2$) to the standard error $\sqrt{\frac{1}{n_1-3}+\frac{1}{n_2-3}}$. If this ratio is 2 or greater the difference is significant. The standard error may be calculated from the square root of the sum of the reciprocals of the number of samples in each correlation, minus 3. In the present case, where the number of samples is 11, $n-3=8$ and the expression for the standard error of Z becomes $\sqrt{\frac{1}{8}+\frac{1}{8}} = .500$. Substituting the values from Table X, we find, Table XI, that there is a significant difference between the correlation constants obtained with the use of bromate and malt, and the value yielded by formula No. 1 plus 40 cc. of potato extract. The difference between the constants for the malt-bromate method and simple formula No. 1 is not significant, while in the presence of sugar and white potato, the difference occasioned by the introduction of diastatic malt is still further removed from significance. Apparently, with the exception of the formulas without sucrose, and without malt but with potato, approx-

TABLE XI

TEST OF SIGNIFICANCE OF DIFFERENCE BETWEEN THE COEFFICIENTS

r	Z	Z_1-Z_2	$\sqrt{\frac{1}{n_1-3}+\frac{1}{n_2-3}}$	$\dfrac{Z_1-Z_2}{\sqrt{\frac{1}{n_1-3}+\frac{1}{n_2-3}}}$
.954 .620	1.874 .725	1.149	.500	2.298
.954 .734	1.874 .937	.937	.500	1.874
.866 .620	1.317 .725	.592	.500	1.184
.734 .494	.937 .541	.396	.500	.792

imately equal correlations between loaf volume and protein content are obtained by all baking methods.

The conclusions reached in the present study regarding the effect of cooked white potato extract are similar to the results obtained by the author in a previous investigation in which dried mashed potato was added to a series of flours. In the presence of sucrose, the addition of potato improved both color and volume, while loaf volume and flour protein were found to be highly correlated.

References

1. Blish, M. J. Cereal Chem. 5: 158-161. 1928.
2. Fisher, R. A. Statistical methods for research workers. 3rd. ed. Oliver and Boyd, London. 1930.
3. Geddes, W. F. Can. J. Research, 1: 528-558. 1929.
4. Geddes, W. F. and Winkler, C. A. Can. J. Research, 3: 543-559. 1930.
5. Harris, R. H. Can. J. Research, 6: 54-67. 1932.
6. Harris, R. H. Cereal Chem. 8: 47-63. 1931.
7. Harris, R. H. Cereal Chem. 8: 113-133. 1931.
8. Jorgenson, H. Cereal Chem. 8: 361-374. 1931.
9. Larmour, R. K. Cereal Chem. 7: 35-48. 1930.
10. Larmour, R. K. and MacLeod, A. G. Sci. Agr. 9: 477-490. 1929.
11. MacLeod, A. G. M.Sc. thesis. University of Saskatchewan, Saskatoon, Sask. 1929.
12. Schnelle, F. Wiss. Arch. Landw., Abt. A. Pflanz. 1: 471-555. 1929.

SNOWFALL IN MONTREAL[1]

By R. De L. French[2]

Abstract

This paper presents a study of the snowfall records of Montreal from 1875 to 1931. The conclusion is reached that the decrease in recorded snowfall is probably due to increase in the city's heat radiation, of which a rough estimate is offered. A method for predicting future seasonal snowfalls is suggested. The distribution of snowfall by months and by storms is investigated, and data concerning the length of the snowy season and the intensity of snowfalls are presented.

Snow interferes with transportation, and embarrasses the contractor who must operate in the winter. The growth of motor travel has made it necessary for cities to clear their streets and it is probable that before many years all main highways will have to be kept passable for motor vehicles throughout the winter. Construction operations are no longer halted by low temperatures, but are and always will be hindered by snow.

Methods of removing and disposing of snow are well developed and fairly satisfactory, though progress is constant. The essence of all these methods is organization, so that the system can be put in operation at short notice when snow begins to fall, and continue to function smoothly so long as the necessity exists. However, there appears to be no way in which this highly perfected, and, generally speaking, efficient organization can determine in advance the approximate scope of its labors. There is no hope that the date, duration and magnitude of every snow storm to come can be predicted, but the possibility that a study of records of past snowfalls might yield useful data certainly exists.

An attempt has been made herein to find rational answers to questions such as these:

(*a*) Can the total snowfall in any future winter be foretold accurately enough so that the prediction may be of value?

(*b*) Which months have heavy snowfalls, which light, and how great are these falls?

(*c*) How often do storms of various durations occur?

(*d*) What is the usual length of the snowy season, when does it begin and when end?

(*e*) On how many days in each month during the winter may snow be expected to fall?

(*f*) What are the frequencies of various rates of snowfall lasting one day, lasting two days, etc.?

(*g*) How much snow may be expected to fall in one day, in two days, etc.?

Records Used

The records considered are those taken at the McGill University Observatory. Previous to 1875 meteorological observations were not regularly made in Montreal, and few of the early data survive.

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Measurements of heavy snowfall, say six inches or over, are made on the western half of the University campus, a substantially level field of between four and five acres. Although this field is partly surrounded by trees and buildings, these are so low that they cannot have any very marked sheltering effect on the central part of the area, where depth measurements are made. Light snowfalls are measured adjacent to the Observatory building. The choice of the observing point is governed largely by conditions during the storm, every effort being made to assure the representative character of the measurements.

Total Snowfall

Total snowfall is reported by the meteorological service in inches per calendar year; each annual record includes parts of two winters. Such disjointed records obviously do not present as accurate a picture of actual conditions as do records for each winter as a unit, *i.e.*, for the period commencing in October of one year and ending in April of the following year. The records of snowfall on which the studies of this paper are based were therefore rearranged in this way. Only storms during which at least 0.1 inch of snow fell are included, this being the usual meteorological fraction.

In Fig. 1, upper curve, the total snowfalls for each winter from 1875-76 to 1930-31 are plotted from the data in Table I. Although there is a wide variation in snowfall from winter to winter, the graph suggests that there is a distinct downward trend throughout the period covered. This is noticeable in the graph of the 10-year moving average, and is most clearly shown by the line marked "Trend." The wavy character of the moving average line also suggests a periodicity in seasonal snowfalls to which reference will be made later. The "Trend" line is located according to the method of least squares, that is, so that the sum of the squares of the deviations of the various points from it is reduced to the minimum, and therefore it is the most probable straight line which can be drawn through this group of points.

The downward slope of the trend indicates that the seasonal snowfall recorded in Montreal has been decreasing since 1875-76, or, if there has been a lesser downward slope during the past few years, this change has not yet become sufficiently pronounced to overcome the downward trend of the records in the earlier years. The reasons for this apparent decrease in snowfall are largely a matter of opinion, but it will be useful to speculate a little about them.

Perhaps the records of later seasons do not represent the true snowfall, or perhaps there has been a real decrease in recorded snowfall. Faulty records may be due to the fact that the reporting station has been unfavorably influenced by the erection of buildings and the like. The Montreal station has probably been so influenced, but it is difficult indeed to believe that the average decrease in snowfall of about one-half inch per season can be caused entirely by such factors.

A much more likely explanation is that, as the density of population around the station increases with the growth of the city, more and more heat is radiated to the atmosphere, tending to melt or even to evaporate some of the snow

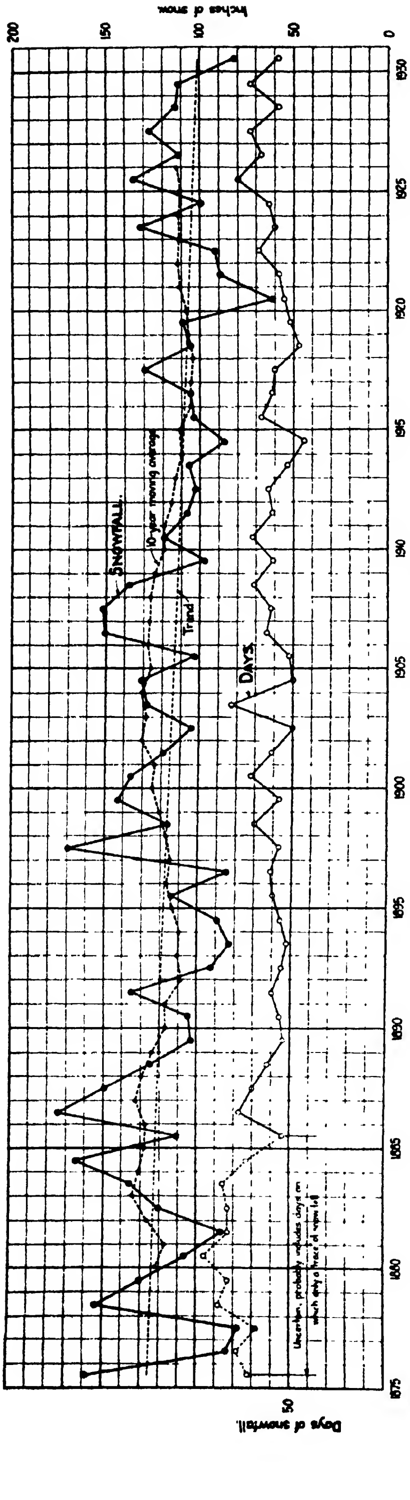

FIG. 1. *Montreal snowfall by winters.*

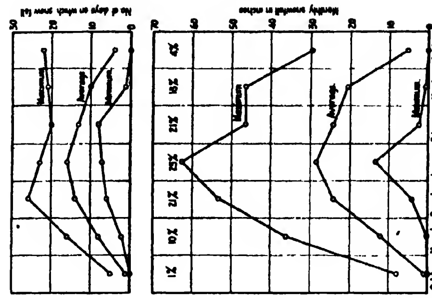

FIG. 4. *Monthly distribution of snowfall and days of snow per month in Montreal.*

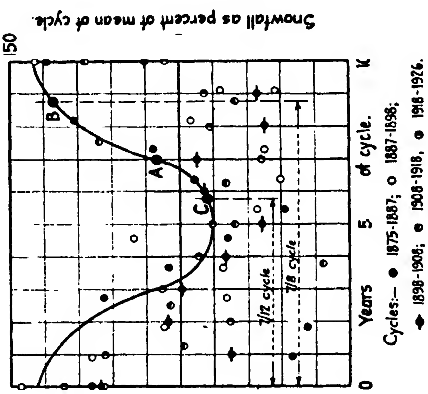

FIG. 3. *Cyclic variation of seasonal snowfall in Montreal.*

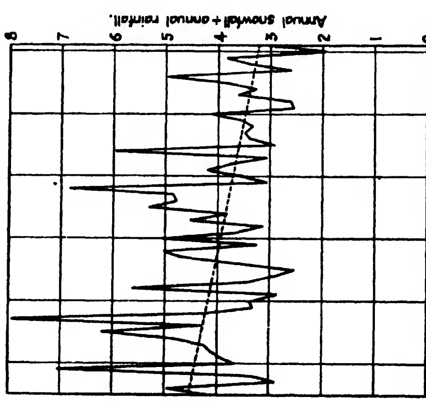

FIG. 2. *Ratios of annual snowfall to annual rainfall in Montreal.*

TABLE I

SNOWFALL IN MONTREAL

SUMMARY OF MONTHLY SNOWFALL, 93

Winter	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Total	Winter	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Total
1875–76	0.0"	21.7"	24.2"	27.4"	22.5"	45.6"	12.0"	158.7"	1903–04	1.5"	5.4"	38.7"	38.3"	21.5"	15.9"	6.5"	127.8"
1876–77	1.0	0.1	23.6	23.3	3.6	22.4	10.2	84.2	1904–05	0.0	10.6	30.3	45.6	38.9	2.7	2.5	130.6
1877–78	5.4	5.1	4.3	30.5	10.2	19.4	2.3	78.2	1905–06	1.0	13.3	28.7	15.2	18.4	18.3	8.2	103.1
1878–79	0.1	14.6	32.8	39.5	27.4	32.6	6.9	153.9	1906–07	0.5	22.4	40.5	22.8	26.7	19.8	17.8	150.5
1879–80	0.0	16.8	37.4	16.3	26.0	25.1	8.6	130.2	1907–08	0.0	14.2	31.2	44.5	40.6	12.2	9.2	151.9
1880–81	3.1	12.7	17.6	26.5	7.3	39.1	0.4	107.9	1908–09	0.0	13.3	53.2	22.1	20.3	20.9	8.9	138.8
1881–82	0.1	11.8	4.4	28.2	23.2	15.3	3.2	86.7	1909–10	0.0	9.4	13.2	30.5	37.8	5.1	1.2	97.2
1882–83	0.0	1.0	39.8	20.0	17.2	35.5	6.7	120.2	1910–11	0.5	8.9	24.9	17.0	31.6	32.8	4.2	119.9
1883–84	0.0	12.1	25.5	44.2	29.3	20.9	3.9	135.9	1911–12	1.5	8.9	12.8	30.6	36.7	14.8	2.2	107.5
1884–85	0.5	5.0	35.0	21.5	43.5	29.1	29.8	164.4	1912–13	0.0	22.1	14.1	22.5	22.4	19.0	2.3	102.5
1885–86	2.8	14.4	36.5	17.4	10.3	26.5	2.8	110.7	1913–14	0.1	4.7	27.5	31.2	14.9	23.0	4.5	105.9
1886–87	0.5	36.1	22.4	50.1	34.1	31.1	0.0	174.3	1914–15	0.6	20.3	27.8	15.6	17.7	4.6	0.0	86.6
1887–88	3.1	25.9	24.9	33.6	30.0	25.2	7.1	149.8	1915–16	0.0	2.0	25.9	17.7	28.1	22.8	6.7	103.2
1888–89	7.8	11.0	17.6	40.5	32.2	15.3	0.1	124.6	1916–17	0.2	3.4	19.4	40.2	21.1	19.0	2.0	105.3
1889–90	0.8	15.6	13.2	31.3	27.4	11.7	3.0	103.0	1917–18	0.0	12.0	20.9	34.0	34.5	26.1	2.1	129.6
1890–91	0.0	8.8	32.3	21.0	18.7	16.3	7.1	104.2	1918–19	0.0	3.3	18.5	16.8	18.6	43.2	4.4	104.8
1891–92	1.5	3.5	12.0	39.7	36.4	34.6	7.2	134.9	1919–20	0.0	10.7	11.2	29.4	29.3	27.9	0.7	109.2
1892–93	0.0	22.1	12.3	22.4	21.1	6.1	8.4	92.4	1920–21	0.2	10.9	21.4	13.6	9.1	6.1	0.3	61.6
1893–94	0.0	5.8	40.4	19.2	9.1	7.4	1.2	83.1	1921–22	0.1	20.6	13.5	20.6	19.4	6.9	8.2	89.3
1894–95	0.0	11.0	23.0	24.9	24.7	5.6	0.0	89.2	1922–23	3.5	1.9	15.1	22.5	23.6	24.4	1.0	92.0
1895–96	0.8	12.7	12.0	20.7	25.9	39.5	3.2	114.8	1923–24	0.0	15.0	23.6	35.5	26.4	14.0	17.1	131.6
1896–97	0.0	5.9	10.8	26.1	16.5	23.7	1.9	84.9	1924–25	0.0	5.3	16.4	41.2	12.2	16.9	7.6	99.6
1897–98	0.0	18.9	39.2	62.7	46.3	0.9	0.7	169.7	1925–26	3.2	10.1	25.2	18.2	31.1	36.0	11.7	135.5
1898–99	0.0	15.7	20.9	25.1	9.1	43.7	1.9	116.4	1926–27	0.6	15.1	35.4	19.4	34.7	5.8	0.3	111.3
1899–1900	0.0	2.4	24.9	36.6	31.5	46.4	0.7	142.5	1927–28	0.0	6.9	34.4	37.3	14.1	23.0	11.8	127.5
1900–01	0.0	34.8	25.2	27.1	22.4	26.0	1.3	136.8	1928–29	0.0	11.8	13.0	29.4	21.5	16.2	11.0	102.9
1901–02	1.0	29.2	15.1	26.6	34.5	9.4	3.4	119.2	1929–30	0.3	3.8	41.4	28.8	11.4	12.3	3.3	101.3
1902–03	0.0	5.9	31.3	33.5	26.2	3.1	4.3	104.3	1930–31	0.0	5.4	12.5	31.8	23.7	8.3	0.0	81.7
									Maximum	7.8	36.1	53.2	62.7	46.3	46.4	29.8	174.3
									Minimum	0.0	0.1	4.3	13.6	3.6	0.9	0.0	61.6
									Mean	0.8	12.0	24.1	28.7	24.3	20.6	5.3	115.8

when it reaches a zone not far above the earth. Such heat radiation also increases the capacity of the air to absorb moisture, but presumably has little or no effect on winds tending to dissipate the heat. Since most snow falls when temperatures are close to 32° F., a rise of a degree or two in atmospheric temperature may determine the nature of the precipitation, whether snow or rain.

Total precipitation as measured at Montreal has been reasonably uniform over the period for which records are available. Wet and dry years offset one another so that the general trend of the records shows little tendency toward either increase or decrease. If total precipitation remains nearly constant, then decrease of snowfall should be accompanied by increase of rainfall, and a decrease in the ratio of annual snowfall to annual rainfall would indicate both a decrease in snowfall and an increase in rainfall. This argument holds rigidly only if a unit depth of snow is equivalent to a constant depth of rain. This is not strictly true, but since one winter's snowfall is the sum of many small falls of all kinds of snow, wet and dry, the mean rainfall equivalent of unit snowfall varies little from season to season.

In Fig. 2 the ratios of annual snowfall to annual rainfall in Montreal for the past 57 years have been plotted. It is quite apparent that these ratios are decreasing; the trend of the decrease being shown by the straight line, which has been located by the methods of least squares.

These facts all lead to the conclusion that the explanation of Montreal's decreasing recorded snowfall lies in the increasing melting of aerial snow by heat radiated from the city. This conclusion is fortified by the fact that snowfall records from areas where natural conditions obtain do not show any such consistent decrease.

It is quite impossible to make any really valuable estimate of the heat radiation of a city; it is thought that in this latitude and type of climate it may easily reach 10,000 B.t.u. per person per hour during cold weather, which is equivalent to 500,000 B.t.u. per hour per acre with the rather moderate population density of only 50 persons per acre.

Short-time Cycles

In addition to the probable long-time cycles of variation in seasonal snowfall, shorter cycles are apparent from the graph of Fig. 1, and are shown in Table II. For the sake of comparison, records from Ottawa and from Quebec are also included in this table.

The lengths of the cycles agree fairly well. There is no marked peak in the Ottawa records between 1887 and 1908, and none in the Quebec records between 1882 and 1908, periods of 21 and 26 years, respectively. The fact that these records are for calendar years and not for winters may mask the peaks in some degree; this effect is noticeable in the Montreal records. The mean length of this short-time cycle appears to lie between 10 and 11 years, which agrees roughly with the sunspot cycle of 11.2 years, with which other meteorological phenomena are frequently correlated, but one should not give this coincidence much emphasis.

TABLE II

SHORT-TIME SNOWFALL CYCLES IN MONTREAL, OTTAWA AND QUEBEC

Montreal		Ottawa		Quebec	
Winter	Years, peak to peak	Year	Years, peak to peak	Year	Years, peak to peak
1875–76		1876		1874	
	11		11		8
1886–87		1887		1882	
	11		11		
1897–98		1898 ?		? ?	26
	10		10		
1907–08		1908		1908	
	10		9		9
1917–18		1917		1917	
	8		9		11
1925–26		1926		1928	
Mean	10		10		11

In Fig. 3 is given a further analysis of each of the five cycles into which the records of seasonal snowfall in Montreal have been divided. Ten years has been adopted as the length of the basic cycle; ordinates have been evenly interpolated between 0 and 10 for cycles differing in length from 10 years. The curve in the figure is located so that when it is used in predicting future snowfalls, the results are more likely to be in excess of the truth than deficient, *i.e.*, so that the predictions are on the safe side.

Prediction of Probable Future Seasonal Snowfalls

Predictions of future seasonal snowfalls based on the foregoing data cannot be less accurate than the blind guesses which must otherwise be made.

To illustrate the method of prediction suggested, assume that the probable snowfall for the winter of 1932-33 is desired. This is seven years after the beginning of the 1925-26 cycle. The mean decrease in seasonal snowfall during a ten-year cycle is about 4.5 in., as read from the "Trend" line of Fig. 1. The mean seasonal snowfall for the cycle beginning in 1917-18 and ending in 1925-26, as computed from Table I, is 105.9 in. Therefore the probable mean seasonal snowfall for the cycle including the winter of 1932-33 is $105.9 - 4.5 = 101.4$ in. From Fig. 3, Point *A*, the seasonal snowfall in the seventh year of a 10-year cycle may be expected to be not more than 106% of the mean for the cycle. Then the probable maximum value for the snowfall during the winter of 1932-33 is $101.4 \times 1.06 = 107.5$ in.

The length of the cycle beginning in 1925-26 is unknown, but was assumed to be 10 years. If it were assumed at eight years, the prediction just made would be increased in the ratio of 1.37 to 1.06 (Fig. 3, Point *B*), or by about 29% to 139.0 in. On the other hand, if 12 years were assumed as the length of the cycle, the prediction would be reduced to the ratio of 0.92 to 1.06 (Fig. 3, Point *C*), or by about 13% to 93.5 in. The probability of the length of a cycle varying this much is not great, if past records are criteria.

If snowfall predictions for 1887-1931 are made from the 1875-1887 cycle by the method just outlined, the errors shown in Table III result.

TABLE III

SUMMARY OF ERRORS IN SNOWFALL PREDICTIONS, 1886–87 TO 1930–31

Sign	No. of errors	Per cent error		
		Maximum	Minimum	Mean
–	14	19.5	0.8	6.4
+	35	96.0	0.8	24.3
+ and –	49	—	—	15.5

Standard deviation = 3.9

Thirty-two of the 49 predictions are within 20% of the true value; this is thought to be a reasonable limit for work of this kind. Although this is by no means a conclusive test of the accuracy of the method, it does serve to show that it offers possibilities of usefulness.

Monthly Distribution of Snowfall

The distribution of snowfall month by month is shown in Table I; at the foot of the table maximum, minimum and mean values are given. These data are also presented in graphical form in Fig. 4. The figures across the top of the lower part of the chart give approximate mean percentages of the total seasonal snowfall occurring in each month.

Records for September and for May have been omitted from this chart as well as from Table I because there are only occasional snowfalls in these months. Since regular observations have been made in Montreal, early snowfalls and late ones have been reported as shown in Table IV.

TABLE IV

EARLY AND LATE SNOWFALLS IN MONTREAL

Date	Snowfall, in.
Sept. 1912	0.1
Sept. 1913	0.1
May 1876	0.3
May 1878	1.0
May 1882	0.5
May 1889	0.1
May 1909	0.1

Distribution and Frequency of Storms of Various Durations

For the purposes of this paper, a storm is considered to mean a single day, or two or more consecutive days, during which 0.1 inch or more of snow fell. Thus, a storm may include several periods of snowfall, separated by intervals of no snowfall, roughly corresponding to the passage of an anticyclonic depression in that succession of highs and lows that dominate our weather.

One-tenth inch of snow may be regarded as too small a fall to be of any consequence, but it should not be forgotten that it is impossible to foretell the probable fall when a storm begins, and that the essence of successful snow fighting is to set the organization functioning promptly at the onset of a storm.

Table V shows the number of days of snowfall in each month since the beginning of the Montreal records; the lower curve of Fig. 1 gives the same information in graphical form. The maximum, minimum and mean number of days of snow for each month are given at the foot of the table, and these data are shown graphically by the curves at the top of Fig. 4.

TABLE V

SNOWFALL IN MONTREAL

NUMBER OF DAYS IN EACH MONTH ON WHICH 0.1 IN. OR MORE OF SNOW FELL

Winter	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Total	Winter	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Total
1875–76	1	16	—	16	15	17	7	72	1903–04	1	14	22	18	15	10	3	83
1876–77	2	5	19	21	11	16	4	78	1904–05	0	4	13	14	13	2	4	50
1877–78	2	8	12	18	12	14	2	68	1905–06	1	5	16	10	9	8	3	52
1878–79	2	12	24	23	16	16	5	88	1906–07	1	9	11	15	18	8	2	64
1879–80	1	8	21	11	16	16	10	83	1907–08	0	7	12	18	11	8	6	62
1880–81	5	15	18	18	11	21	8	96	1908–09	0	6	22	17	11	11	4	71
1881–82	5	13	12	20	13	15	5	83	1909–10	0	7	15	15	17	6	1	61
1882–83	0	5	24	16	16	15	7	83	1910–11	1	7	18	17	12	14	3	72
1883–84	0	8	17	21	20	14	6	85	1911–12	2	9	7	18	13	8	4	61
1884–85	5	10	14	12	11	12	9	73	1912–13	0	10	12	14	16	9	2	63
1885–86	1	7	14	17	11	1	3	54	1913–14	1	2	9	15	9	12	5	53
1886–87	1	15	13	19	15	14	0	77	1914–15	2	8	9	7	11	7	0	44
1887–88	3	13	10	13	14	9	8	70	1915–16	0	5	13	14	19	10	6	67
1888–89	4	6	12	14	15	10	1	62	1916–17	1	5	11	18	13	12	2	62
1889–90	1	4	13	16	11	7	2	54	1917–18	0	9	7	17	15	10	2	60
1890–91	0	6	16	12	14	6	2	56	1918–19	0	3	10	14	9	7	4	47
1891–92	2	5	6	21	13	9	4	60	1919–20	0	6	9	14	13	8	2	52
1892–93	0	11	12	12	9	7	4	55	1920–21	1	12	14	11	12	4	1	55
1893–94	0	6	18	13	9	5	1	52	1921–22	1	13	9	14	12	5	4	58
1894–95	0	11	10	14	12	9	9	56	1922–23	2	4	13	13	13	18	6	69
1895–96	1	5	8	13	16	12	5	60	1923–24	0	5	9	18	10	12	6	60
1896–97	0	7	9	14	10	10	11	61	1924–25	0	5	15	22	10	7	4	63
1897–98	0	5	16	18	13	1	4	57	1925–26	5	2	15	17	14	15	12	80
1898–99	0	6	19	17	11	15	2	70	1926–27	2	12	19	15	15	3	1	67
1899–1900	0	2	11	14	12	15	3	57	1927–28	0	8	11	17	17	13	7	73
1900–01	0	12	17	13	11	18	1	72	1928–29	0	5	8	17	13	8	7	58
1901–02	1	14	11	14	13	6	2	61	1929–30	1	8	26	14	8	13	3	73
1902–03	0	3	16	14	10	4	3	50	1930–31	0	4	12	13	19	10	0	58
									Maximum	5	16	26	23	20	21	12	96
									Minimum	0	2	6	7	8	1	0	44
									Mean	1	8	14	16	13	10	4	65

A tabulation of the distribution of storms of different durations is given in Table VI.

TABLE VI

MONTHLY DISTRIBUTION OF SNOWSTORMS OF VARIOUS DURATIONS IN MONTREAL

Month	Duration of storm in days										
	1	2	3	4	5	6	7	8	9	10	11
Oct.	28	4									
Nov.	90	42	24	9	3	3	1	1			
Dec.	134	84	22	17	14	4	2	1	1		1
Jan.	136	101	36	21	13	3	5			1	1
Feb.	118	92	30	18	8	5	2	2	2		
Mar.	108	64	30	10	5	3	1	1			
Apr.	77	17	8	5							
Total storms	691	404	150	80	43	18	11	5	3	1	2
Total days	691	808	450	320	215	108	77	40	27	10	22
% of total days	25	29	16	12	8	4	3	1	1	0	1

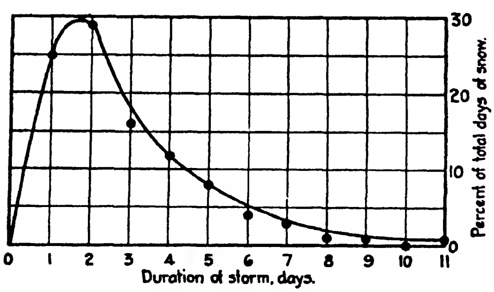

FIG. 5. *Frequency of snow storms of various durations in Montreal.*

A clearer idea of the frequency of storms of various durations is given by Fig. 5, which has been plotted from the data of this table. It will be noted that only about 11% of the total number of days of snow occurs in storms lasting more than five days.

Length of Season of Snowfall

The earliest date of snowfall, excluding those given in Table IV, was October 3; the mean date of the beginning of the snowy season is October 30. Excluding the dates given in Table IV, the latest snow fell on April 30, while winter usually ends about April 17. The interval between the first snowfall and the last of the season varied from 137 to 224 days, with a mean length of 170 days. Table V shows that snow fell on from 44 to 96 days; during an average winter there were about 65 days of snowfall. This is equivalent to saying that snow falls to the amount of at least 0.1 in. on four days out of ten from November 1 to April 15.

Intensity of Snowfalls

A series of curves has been plotted in Fig. 6 from the analysis of 2,862 single days of snowfall, and from records of 1,837 days comprising storms up to and including five days' duration. The records of the shorter storms are not included in those of the longer, except in the case of single-day storms, which are so included.

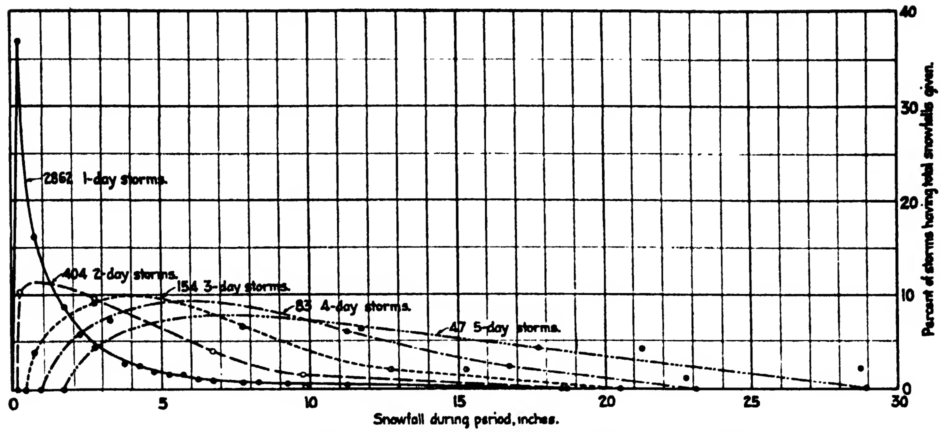

FIG. 6. *Frequency of various storm snowfalls in Montreal.*

TABLE VII

SIX- TO ELEVEN-DAY STORMS IN MONTREAL

Duration, days	Number of records	Snowfall, in.	
		Minimum	Maximum
6	17	4.5	17.4
7	13	1.8	23.5
8	5	8.0	21.3
9	2	15.2	26.0
10	2	14.5	24.6
11	2	13.5	29.4

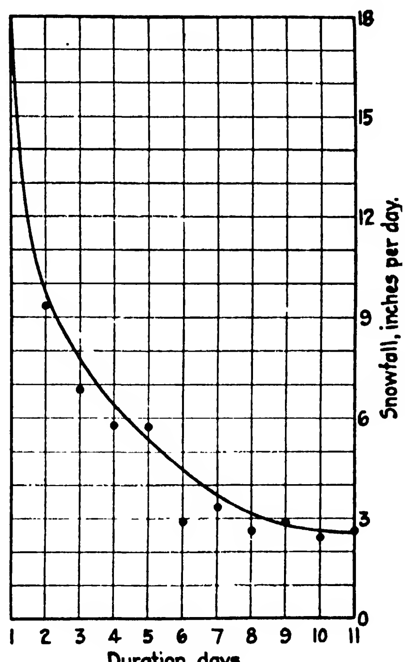

FIG. 7. *Maximum daily snowfalls for storms of various durations in Montreal.*

The points plotted are those which roughly define the envelopes of the groups: other points have been omitted for the sake of clearness. The curves therefore give values which have been exceeded only rarely in the past, and which are equally unlikely to be exceeded in future.

From these graphs, the maximum and the minimum snowfall of record, and the limiting probability of the occurrence of any snowfall between them for any of the storms represented may be determined. The curve for the four-day storms, for example, shows that the falls have ranged between 0.9 and 23.1 in.; a fall of 18 in. occurs in not more than 2% of such storms.

Records relating to storms lasting longer than five days are not numerous enough to be included in Fig. 6. Table VII gives some data regarding such storms. There are no records of storms lasting longer than 11 days.

Maximum daily rates of snowfall for storms of different durations are shown in Fig. 7.

Acknowledgments

The writer wishes to express his thanks to Professor A. J. Kelly, Director of the McGill University Observatory, for supplying the Montreal records, to the Dominion Meteorological Service for those from Ottawa and Quebec, and to Professor James Weir and many others of his colleagues for their interest and helpful criticism.

ON TWO NEPHELINE-SODALITE-SYENITES FROM NEW LOCALITIES IN NORTHERN RHODESIA[1]

By Frank Dawson Adams[2] and Freleigh Fitz Osborne[3]

Abstract

Two specimens of nepheline-sodalite-syenite from Northern Rhodesia are petrologically interesting because of the low content of binary oxides and the presence of an aluminous aegirine. The rocks are similar in chemical composition to lavas found at the north end of Lake Nyassa and probably belong to the same petrographical province.

Introduction

In a recent communication Dr. J. Austen Bancroft, Consulting Geologist of the British South Africa Company, writing from Nkana, refers to a number of discoveries of great scientific interest that are being brought to light through the detailed geological survey of the Company's concession in Northern Rhodesia, which is being actively prosecuted by a large staff of geologists under his direction. These will be described in detail when the work has been completed. Among the newly discovered occurrences, which promise to be of special interest to petrographers, are large areas of alkaline rocks. During the months of November and December of last year two widely separated areas of these were found. The rocks constituting these occurrences, Dr. Bancroft states, are very similar in appearance to the nepheline-bearing rocks, described by Adams and Barlow, in the Haliburton District of Ontario, some of them being rich in sodalite, cancrinite, etc.

The writers have received from Dr. Bancroft two of the first specimens of these rocks which have been collected. This paper presents the results of a petrographical examination of them, which must of course be supplemented by a detailed study of the whole petrographical province as the survey is continued. It serves merely to set forth in some detail the character of these two specimens of Rhodesian nepheline syenites, to show that they are rocks of peculiar interest, and to indicate that a further study of the area from which they are derived will probably bring to light a highly interesting series of comagmatic rocks.

The specimens received from Dr. Bancroft were labelled *A* and *B*, respectively. *A* is a nepheline-sodalite-syenite from a point 40 miles west of Solwezi, and a short distance south of the boundary line between Northern Rhodesia and the Belgian Congo. Solwezi is 80 miles in a westerly direction from Elizabethville.

B is a nepheline-sodalite-cancrinite-syenite from the northeast corner of Northern Rhodesia at a point between the Loangwa River and the border of Nyassaland. It is about 65 miles south of Fort Hill. The two localities are about 500 miles apart.

[1] *Manuscript received April 27, 1932.*
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Nothing is known by the writers concerning the age or geological relations of these igneous rocks. In Fairweather's geological map of Northern Rhodesia, which is reproduced in Krenkel's *Geologie Afrikas* (1, p. 488), the locality from which specimen *A* was taken is indicated as Muschinga schist and Lumagundi schists (Broken Hill schists) in intimate association, while the locality from which specimen *B* was taken is shown as Karru beds but not very far distant from a large occurrence of gneisses and ancient crystalline schists.

Both specimens were evidently taken from outcrops and each shows one weathered surface. While this may be termed a weathered surface, the rocks as a matter of fact show no alteration from the action of the weather. They are perfectly fresh and show no decomposition products, but the surface which has been exposed to the weather resembles that displayed by the nepheline syenites of the Haliburton District in Ontario in that the feldspathic constituents stand out from it in relief, whereas the nepheline, sodalite and other feldspathoids occupy depressed areas as if these minerals had been dissolved away by the action of the weather.

Thirty-two thin sections of the rocks were prepared and examined.

Specimen A. Nepheline-sodalite-syenite (Aegirine-ditroite)

Forty miles west of Solwezi and south of the boundary between Northern Rhodesia and the Belgian Congo

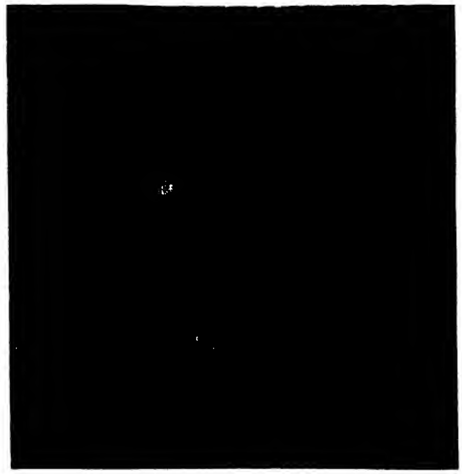
The rock is of medium granularity with four constituents visible on the fresh surface. White feldspar, which shows Carlsbad twinning and a tendency to form radiating groups, is the most abundant constituent. Pale-blue sodalite forms patches interstitial to the feldspar. Nepheline, of a paler blue than the sodalite, forms cores to some areas of sodalite. A pale-green pyroxene is present in fibrous clots. The feldspar is the only constituent showing any tendency toward idiomorphism. This is especially evident on the weathered surface, where the feldspathoids have been removed, and the feldspar laths form a boxwork around the depressions.

Fifteen thin sections of the rock were examined. They show microcline, albite, aluminous aegirine, nepheline, and sodalite, as the principal constituents. Zircon, calcite, magnetite, hematite, cancrinite, perovskite, a hydrated iron oxide, and an unidentified mineral are accessory.

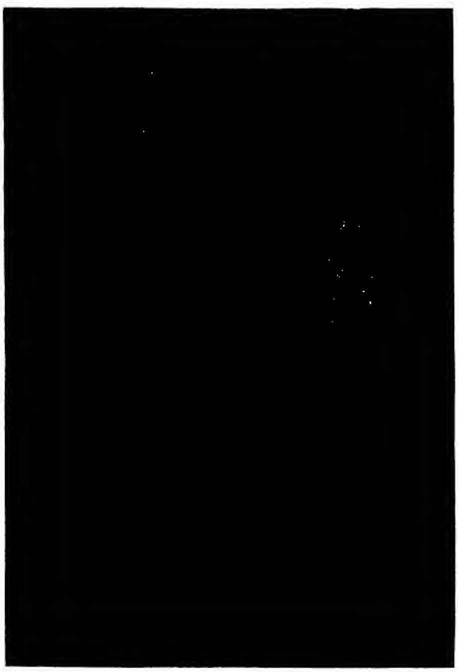
A determination of the proportions of minerals by the Rosiwal method reduced to weight per cent gives: microcline and albite, 52%; sodalite, 21%; pyroxene, 14%; nepheline, 11%; accessory minerals, 2%.

Microcline, the most abundant constituent, tends to be tabular parallel to crystal axis *a* and to form rudely radiating groups, but does not show good crystal outline. The cores of some of the microcline anhedra are somewhat clouded, but the margins are clear. Much of it has been replaced by albite. In some grains the replacement is along irregular zones as shown in Fig. 1, but in others only the margins have been attacked. Many of the small anhedra of albite show no multiple twinning, but most of the larger grains are well twinned. Where the microcline has been almost entirely replaced, the

2A



2B



1

3

FIG. 1. *Radiating aggregate of microcline partly replaced by albite in the ditroite.* (*A*) ×23. *Nicols* ×.

FIGS. 2A AND 2B. *Specimen B. Fig. A shows the common relationship in which aegirine* (*middle of field*) *is separated from biotite* (*black*) *by a zone of light minerals. B shows an exceptional arrangement in which an anhedron of aegirine is surrounded by biotite and both enclosed within microcline microperthite.* ×23.

FIG. 3. *Ilmenite rods within magnetite. From B. Reflected and oblique light.* ×23.

albite is lamellar and in somewhat diverging groups so that it resembles the cleavelandite found in granite-pegmatite dykes. Only a little albite appears to have formed away from microcline.

Aluminous aegirine, which does not show good crystal boundaries, is present in isolated rods, irregular bundles, or fibrous aggregates and appears to replace feldspar. It is unusual in that it is practically colorless, in this respect resembling rhombic pyroxene from which it differs, however, in possessing high indices and birefringence It shows very faint pleochroism in brown and green in the prismatic sections. The section normal to c shows nearly central emergence of α and a parting (not due to twinning) parallel to 100, bisecting the angles between the traces of the prismatic cleavage and at right angles to the trace of the optic-axial plane.

The lower index of refraction, α, determined by immersion is 1.72 and $\gamma-\alpha$, estimated in thin section from a grain showing central emergence of the optic normal, is about .03. The extinction angle, $\alpha \wedge c$, is 7°. It is optically negative with an optic-axial angle of 65° and a marked dispersion $\rho > \nu$. The strong dispersion causes incomplete extinction in white light so the color changes from blue to brown on going through the position of extinction. In contact with perovskite it is an intense canary yellow with only appreciable differential absorption.

The chemical analysis of the rock given below as I shows an excess of Al_2O_3 over Na_2O, K_2O and CaO. Part of the Al_2O_3, as $NaAl(SiO_3)_2$, has undoubtedly replaced part of the Fe_2O_3 of the aegirine molecule. Jadeite occurs in omphacite. It is probably this molecule in the pyroxene that accounts for the value of α .05 below that of aegirine proper, a birefringence of about half of that of aegirine, and the absence of strong pleochroism.

Nepheline is found in water-clear or only slightly clouded grains interstitial to the feldspars. It is replaced around its margins by large individuals or granular aggregates of sodalite or a related mineral.

The sodalite contains small myrmekite-like anhedra of nepheline, microcline and albite which are parallel to adjacent grains of the corresponding minerals. Similar inclusions of microcline and albite are not found in nepheline, which suggests that the sodalite was formed at the expense of nepheline, microcline and albite along grain boundaries, particularly near nepheline. Some parts of the sodalite are a pale canary yellow but only where they are very close to the pyroxene and perovskite.

Zircon occurs in somewhat rounded and equidimensional grains. It is not appreciably radioactive judged by its effects on adjacent minerals. Perovskite is present in rounded isotropic grains of very high index. The cores of the larger grains are colorless, grading to light red at the margins. The smaller anhedra are entirely red and appear to be more radioactive than the larger colorless grains judging from the effect on adjacent minerals. Magnetite is in minute octahedra found near the pyroxene. Larger aggregates of magnetite, hematite, and magnetite oxidized to hematite, are interstitial to the silicate minerals. Careful search of polished surfaces failed to show any metallic sulphide. Uniaxial carbonate is found in two parageneses: in some places it is

older than, and surrounded by, pyroxene, in others it replaces feldspar. Where it is associated with the pyroxene it shows grains of a dark red, strongly absorptive mineral projecting into it from the margins. The mineral could not be identified definitely, but is probably an iron oxide. This suggests that the carbonate is iron-bearing, but it could not be isolated for definite determination. Three very small grains of an unidentified mineral were found in the 15 sections. The mineral has high indices of refraction and birefringence, a large optic-axial angle, a negative sign, and extinction angle of 45°. The rock is an aegirine-ditroite.

Specimen B. Nepheline-sodalite-cancrinite-syenite

Northeast corner of Northern Rhodesia, between the Loangwa River and the border of Nyassaland

The rock is coarse in grain. It is composed of microcline, albite, nepheline, sodalite, cancrinite, biotite, pyroxene, with smaller amounts of magnetite, ilmenite, hematite, pyrite, chalcopyrite, zircon and calcite.

The feldspars are pale mauve in color and show only simple twinning. Black biotite and pyroxene are the ferromagnesian minerals. Blue sodalite and nepheline with honey-yellow cancrinite are interstitial to the feldspars.

The microcline in thin sections is water-clear and shows the usual quadrille and Carlsbad twinning but is replaced along irregular branching veins by albite. This injection perthite is in marked contrast to the more regular structure of the microperthite formed by unmixing of a solid solution. In addition, small unorientated grains of albite occur in microcline near the grain margins, along fractures, and along the Carlsbad twinning junctions.

The nepheline is present in large grains completely or partly altered to cancrinite and sodalite. The alteration appears to start at the margins and proceed toward the centre of the grains, although, in some places, fractures in the nepheline have localized the formation of cancrinite. The sodalite, which also appears to have been replaced by the cancrinite, has some inclusions of natrolite.

Pyroxene, optically similar to that from the aegirine-ditroite, occurs in small amounts. In one thin section, it is surrounded by biotite apparently formed by reaction; in others, however, it is found without any reaction rim. See Figs. 2A and 2B.

Biotite, which is the abundant mafic mineral, is highly differentially absorptive and changes from a very dark green to an olive shade as the stage is rotated. The crystals are idiomorphic and appear to be of late development because in one place unbroken biotite is found in a minor shear zone, marked by the broken feldspars.

Magnetite, magnetite with intergrown ilmenite (Fig. 3), ilmenite, hematite, pyrite, and chalcopyrite are interstitial to the silicates. The pyrite appears to have replaced the oxides and has in turn been replaced by chalcopyrite (a few minute grains) and a hydrated iron oxide. Zircon is found in very small grains, but no perovskite was noted. Calcite replaces feldspar and, in some places, grains of calcite localized rims of cancrinite in nepheline.

Chemical Composition

The aegirine-ditroite (Specimen *A*) was analyzed for the writers by Miss Mary G. Keyes, formerly chemical assistant to Dr. Henry Washington at the Geophysical Laboratory, Washington, D.C. The results of this analysis are given as No. I in Table I—with it are given analyses of three other rocks for

TABLE I

COMPARISON OF ANALYSIS OF SPECIMEN *A* WITH ANALYSES OF OTHER ROCKS

Constituent	I	II	III	IV
	Per cent			
SiO_2	56.31	57.87	56.74	62.02
TiO_2	Tr	.48	.40	.31
Al_2O_3	19.89	18.46	19.32	18.71
Fe_2O_3	4.28	4.63	2.37	4.30
FeO	.29	.40	1.65	.10
MnO	.16		.07	.15
MgO	.26	.61	.27	.40
CaO	.74	1.03	1.98	.86
Na_2O	8.33	8.83	8.05	6.90
K_2O	5.75	5.74	5.88	4.93
H_2O+	.68	.90	1.12	.80
H_2O-	.13	.70	.32	.31
CO_2	1.27		1.50	none
P_2O_5	n. d.	.14	.03	.24
ZrO_2	.16		.02	.06
SO_3	.19		.12	.02
Cl	1.13	.62		none
BaO	Tr		.16	.02
	99.57	100.41	100.17	100.13

I. *Ditroite. Specimen A. Analyst, Mary G. Keyes.*
II. *Phonolite, Patagwa River, near Utanjilva, Nyassa. Analyst, E. Lehmann,* (*2, p. 107*).
III. *Cancrinite syenite, Beaver Creek, Uncompahgre quadrangle, Col. with SrO .12 and S .05. Analyst, G. Steiger* (*3, p. 295*).
IV. *Trachyte, Puu Anahulu, Hawaii. Analyst, H. S. Washington* (*4, p. 108*).

TABLE II

THE NORM OF NO. I

Constituent	%	Constituent	%
Orthoclase (Or)	34.47	Sodium Carbonate (Ne)	1.38
Albite (Ab)	47.68	Zircon (Z)	.18
Anorthite (An)	none	Olivine (Ol)	.56
Nepheline (Ne)	2.27	Magnetite (Mt)	.93
Corundum (C)	3.47	Hematite (Hm)	3.68
Halite (Hl)	1.76	Calcite (Cc)	1.30
Thenardite (Th)	.28		
			97.96

comparison. It is interesting to note that analysis II, of a phonolite from near Utanjilva, Nyassaland, is almost identical with that of aegirine-ditroite (Specimen *A*) from Northwest Rhodesia. The two localities must be 500

miles or more apart but as both are rather unusual in chemical composition, it would suggest that they belong to the same petrographical province or to two petrographical provinces which resemble one another closely.

The norm of No. I is shown in Table II. The low summation of this norm is due to the omission of oxygen taken from Na_2O to form NaCl and to the water which must be added.

It will be noticed that this rock is distinctly "non modal", that is to say, the norm differs very considerably from the mode or actual mineral composition as seen under the microscope. This same divergence has been noted by Washington in the case of the Trachyte from Puu Anahulu in Hawaii, his analysis of which is given as IV in Table I, and which resembles the rock now under consideration although considerably higher in silica. Both analyses have been very carefully made and checked and there is every reason to believe that they are correct and, as Dr. Washington states, this non modal character in both of these rocks, which are similar in character, is "difficult to understand". It may possibly be connected in some measure with the peculiar composition of the pyroxene present whose chemical composition is as yet unknown but which will be further studied.

Comparison with the Syenite of Ditro

The abundance of potassic feldspar shows that the rocks are related to the Foya rather than the Litchfield or Canada type of nepheline syenite.

An examination of the nepheline syenites in the petrological collection at McGill University shows no one that is identical with either specimen from Africa. The aegirine-ditroite resembles to some extent the finer-grained variety of ditroite from Ditro, except that the feldspar of the Ditro rock is somewhat more glassy in appearance, and the ferromagnesian minerals are black, whereas those of the African specimen are pale green. Under the microscope the texture is different: the sodalite of the ditroite is in equidimensional anhedra; the aegirine is a very strongly absorptive variety, and has a reaction rim of the blue-green amphibole known as hastingsite; and titanite is abundant.

The feldspar of the coarser African rock resembles in color that of the Laurdalite from Laurvig but is, of course, microcline.

References

1. Krenkel, E. Geologie Afrikas, Part 2. Borntraeger, Berlin. 1928.
2. Lehmann, E. Das Vulkangebiet am Nordende des Nyassa als magmatische Provinz. Z. Vulkanologie, Ergänzb. IV. 1924. See Johannsen, A. A descriptive petrography of the igneous rocks. v. 1. p. 115. Univ. Chicago Press. 1931.
3. Washington, H. S. Washington's Tables, p. 295, No. 13.
4. Washington, H. S. Petrology of the Hawaiian Islands. II. Hualalai and Mauna Loa. Am. J. Sci. 6: 100-126. 1923.

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THE FLOW OF HEAT THROUGH PLATES[1]

BY R. RUEDY[2]

Abstract

The speed is calculated with which the steady flow of heat is established in a slab of uniform temperature after one boundary plane has been suddenly brought to a higher temperature, or when the temperature of both planes is changed. In both cases the flow of heat may be expressed by means of simple theta functions, and the law of approach to the steady state may be used for determining the diffusivity of the material. When one boundary plane undergoes sinusoidal variations in temperature while the other is maintained at a constant level, a finite thickness is found for which, in the steady state, the heat flowing in or out during one half-cycle reaches its highest value.

The Steady State in the Plate Method

When the "plate" method is used for measuring the heat conductivity of solid bodies, one side of a slab is more or less suddenly brought into contact with a source of heat at constant temperature, while the other side is kept cool. Heat begins to flow through the slab, and when a steady state is reached, the coefficient of heat conductivity, k, is determined. Losses which would be caused by radiation, conduction and convection through the rest of the boundary surface are prevented with the aid of a guard ring surrounding the slab. How closely the heat conductivities may be ascertained depends on the accuracy with which the thickness of the plate, the temperatures of the two walls, and the heat energy, Q, furnished to one side or drawn from the colder end, may be measured. The coefficient k is determined from the formula

$$k(c.g.s.) = \frac{Q(\text{watts}) \times b(\text{cm.})}{4.184A(\text{sq. cm.}) \times (v_1 - v_0) \text{ deg. C.}},$$

where $v_1 - v_0$ is the temperature difference maintained between the two boundary planes. As it is not practicable, in general, to measure the thickness to within less than 0.1 mm. it is desirable to test slabs at least 5, or better, 10 mm. thick in order to keep the possible error below 2%. For a temperature difference of 15° C., an interval over which k may be considered as being constant, an

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Contribution from the National Research Laboratories, Ottawa, Canada. This paper was read at the meeting of the Royal Society of Canada, May 1932.

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error of 0.2° C. means an uncertainty of 1.3%. The energy, Q, furnished, computed from the current measured to within 0.002 amp. and the voltage read to within 0.04 volt, is known with an accuracy of between $\frac{1}{2}$ and 1%, so that the uncertainties entering into the value of k will not be below $\pm 2\%$ (2). The precision may be improved by using thicker plates, but when many measurements have to be made the question arises as to how much longer it will take for the steady state to be established. While it is also possible to increase the temperature difference between the hot and the cold plane, the change of k with temperature may introduce new errors.

When at the beginning of the experiment the temperature in a slab of thickness b, specific heat c and density s, is a function, $f(z)$, of the distance z from the cold wall of the slab, and is equal to zero at time t equal to zero, while the ends are kept at the temperatures $F_0(t)$ and $F_b(t)$ during the interval from $t=\lambda$ to $t=t$, then the temperature v at any distance z is at any instant t represented by the following formula (1, p. 69).

$$v = \frac{2}{b} \sum_{n=1}^{\infty} \epsilon^{-n^2 \frac{\pi^2}{b^2} \varkappa t} \sin n \frac{\pi}{b} z \left[\int_0^b f(z) \sin n \frac{\pi}{b} z dz + n \frac{\pi}{b} \varkappa \int_0^t \epsilon^{+n^2 \frac{\pi^2}{b^2} \varkappa \lambda} (F_0(\lambda) - (-1)^n F_b(\lambda) d\lambda \right],$$

where $\varkappa = \dfrac{k}{cs}$ is the diffusivity of the material.

In the simplest case the temperature is constant throughout the thickness, and one side of the slab is maintained at this temperature which may, therefore, be chosen as the zero point so that $f(z)$ becomes equal to zero. The first term in the brackets in the general equation vanishes, so that $F_b(z) = V$ being the constant temperature at the hot side, the relation reduces as follows:

$$v = -2 \frac{\pi}{b^2} \varkappa V \sum_{n=1}^{\infty} \epsilon^{-n^2 \frac{\pi^2}{b^2} \varkappa} n \sin n \frac{\pi}{b} z \int_0^t (-1)^n \epsilon^{+n^2 \frac{\pi^2}{b^2} \varkappa \lambda} d\lambda.$$

Integrating and taking into account the fact that

$$\sin \frac{\pi}{b} z - \tfrac{1}{2} \sin \frac{2\pi}{b} z + \tfrac{1}{3} \sin \frac{3\pi}{b} z - \dots\dots = \frac{\pi}{2b} z,$$

the equation becomes

$$v = V \left(\frac{z}{b} + \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{(-1)^n}{n} \epsilon^{-n^2 \frac{\pi^2}{b^2} \varkappa t} \sin n \frac{\pi}{b} z \right).$$

The heat C flowing through unit area at a distance z and time t is obtained by differentiating v with respect to z and multiplying by k.

$$C = -k \frac{V}{b} \left(1 + \sum_{n=1}^{\infty} (-1)^n \epsilon^{-n^2 \frac{\pi^2}{b^2} \varkappa t} \cos n \frac{\pi}{b} z \right)$$

or

$$C = -k \frac{V}{b} \Theta(z/b, q)$$

where θ is the symbol for the theta function and $q = \epsilon^{-\frac{\pi^2}{b^2} \varkappa t}$. The heat directly measured and flowing into the slab from the hot side $z=b$ is therefore

given by the expression

$$C_b = -k\frac{V}{b}\Theta_3(o, q) = C^0\Theta_3(o, q),$$

whereas the heat penetrating into unit area of the cold wall is

$$C_0 = -k\frac{V}{b}\Theta_0(o, q) = C^0\Theta_0(o, q),$$

where C^0 is the heat flow $\left(-\frac{kV}{b}\right)$ in the steady state. The values of these theta functions are listed in convenient recently issued tables (3). As an illustration of the rate at which the steady state is established Table I shows the times in seconds in which certain fractions θ_3 and θ_0 of the final heat flow are reached in the case of a slab of sulphur 3.14 cm. thick (conductivity k, 0.0005; specific heat, 0.17; density, 2.0; and therefore $\varkappa$, 0.00147), or slightly compressed spruce of 16% moisture content, the flow being measured parallel to the grain (specific heat, 0.33; density, 0.4). Under the conditions stated the coefficient of heat conductivity is the same for sulphur and spruce but on account of the smaller value of cs a higher value for $\varkappa$ (0.0038) results for spruce,

TABLE I

TRANSIENT STATE OF HEAT FLOW

$\varkappa\frac{\pi^2}{b^2}t$	$\exp.\left(-\varkappa\cdot\frac{\pi^2}{b^2}t\right)$	θ_3	θ_0	Time in sec. $b = \pi$ cm. Sulphur	Spruce	Air
1.53	0.2176	1.439	0.569	1041	404	8.6
1.95	0.1428	1.286	0.715	1327	515	10.9
2.33	0.0975	1.195	0.805	1585		
2.71	0.066	1.132	0.868	1843		
3.21	0.0400	1.080	0.92	2184		
3.79	0.0250	1.050	0.950	2578	1000	21.2
4.27	0.0140	1.028	0.972	2905	1127	23.9
5.11	0.0060	1.012	0.988	3476	1348	28.5
5.38	0.0046	1.009	0.9908	3660	1420	30.0
6.81	0.0010	1.002	0.998	4633	1797	38.0
7.61	0.0005	1.001	0.999	5177	2008	42.5

and less time is needed for reaching the steady state in which $\theta_3 = \theta_0 = 1$. The times given for sulphur also apply to wood fibre (k, 0.00015; c, 0.31; s, 0.33; $\varkappa$, 0.00146). For any other material they may be found by dividing the figures of the first column by $\varkappa$. As is to be expected, the rate at which heat flows at the beginning from the hot side into the slab is larger than the amount flowing in the steady state because the material must be heated up. Near the cold side, on the contrary, there is practically no heat flowing until $\frac{\pi^2}{b^2}\varkappa t$ or $\varkappa t$, in the present case, exceeds a value of about 0.23; after it has become equal to 0.3, the flow is 0.002 of the final value C_0, at 0.6 equal to 0.074 C^0, at 1.0 equal to 0.3 C^0, etc. Halfway between the two side-walls the number

of calories passing through unit area changes with time according to the expression

$$C_{\frac{1}{2}} = 2C_0 \left(\tfrac{1}{2} - \epsilon^{-4 \frac{\pi^2}{b^2} xt} + \epsilon^{-16 \frac{\pi^2}{b^2} xt} - \epsilon^{-36 \frac{\pi^2}{b^2} xt} + \ldots \right).$$

Here the flow has already reached the fraction 0.63 of its final value at a time when practically no heat has started to flow at the cold end. Each section has its own law governing the velocity with which the steady state is attained, because the parts near the hot plate have to transfer energy to the colder parts, whereas, toward the cold side, where this task is easier, it takes an appreciable time for the heat to arrive. From the moment, therefore, at which in the ideal case one side of the slab is suddenly brought to the desired temperature, until the time when the temperature gradient has become constant throughout the thickness, the shape of the temperature waves penetrating the plate varies from point to point so that no definite velocity of propagation can be assigned to the wave fronts. Moreover, the equation is not meant to apply to minute intervals of time or space, such as the transfer of heat from molecule to molecule, because this exchange is not governed by k, c and s only.

The last column of Table I gives the results for the velocity of heat transmission between an upper hot and a lower cold layer of air (k, 0.000055; c, 0.237; s, 0.00129; $\varkappa$, 0.179). On account of the low density the steady state is rapidly established despite the low heat conductivity k. The figures would not apply if a layer placed between the hot and cold "plates" were considered on account of the finite contact resistance to heat flow between the solid walls and the neighboring air layers. Apart from radiation and convection the quantity of heat flowing in the steady state from 1 sq. cm. of a horizontal plate, kept at temperature v_1, into the air layers below it where the temperature is v_2, is given in cal. per sq. cm. per sec., by the formula

$$C = \alpha_{12}(v_1 - v_2) = 0.000077 \sqrt[4]{v_1 - v_2}\ (v_1 - v_2).$$

The fourth root changes only from 1.8 to 3 when the temperature difference $(v_1 - v_2)$ varies from 10.5 to 81° C., the range over which the formula has been verified (4, p. 54). On going from the solid wall into the air layers, the temperature drops fairly rapidly at first and then assumes the value which it has in the layers farther away from the surface. The thickness of these boundary layers is of the order of 0.4 cm. for air at ordinary pressures. For the range of temperature differences tested the heat passing from the plate into the air has nearly the same value as if the temperature differences were established between air layers 0.3 to 0.4 cm. thick, but the gradient is not linear and the change with time will follow a different law. On the other hand, when a slab of material possessing the heat conductivity k_3 is separated from the hot plate by an air space several mm. wide, the coefficient of heat conductivity k of the assembly is given by the expression

$$k = \frac{1}{\dfrac{1}{\alpha_{12}} + \dfrac{1}{\alpha_{23}} + \dfrac{1}{k_3}} \approx \frac{k_3}{\dfrac{2k_3}{\alpha_{12}} + 1}$$

The higher the value of k_3 the larger will be the change in k_3 but this change is negligible for very good insulators.

As it will take nine times longer, when one plate is three times as thick as the other, to get the same fraction C_b/C^0 of the steady current C^0, 15 to 30 min. should be ample for thicknesses below 1 cm. to arrive at the steady state. If C_b is measured at a few intervals with V maintained constant automatically, the value of k can be checked and the diffusivity determined at the same time.

Slight Annealing or Quenching of a Slab

The same functions appear when the case is treated in which a thin slab at uniform temperature is suddenly plunged into a bath maintained at V° C. The first term in the brackets of the general formula is then the one that remains

$$v = \frac{2}{b} \sum_{n=1}^{\infty} \epsilon^{-n^2 \frac{\pi^2}{b^2} xt} \sin n \frac{\pi}{b} z \int_0^b f(z) \sin n \frac{\pi}{b} z \, dz,$$

or

$$v = \frac{4V}{b} \left(\epsilon^{-\frac{\pi^2}{b^2} xt} \sin \pi \frac{z}{b} + \tfrac{1}{3} \epsilon^{-9 \frac{\pi^2}{b^2} xt} \sin 3\pi \frac{z}{b} + \dots \right),$$

and

$$C = -k \frac{dv}{dz} = \pm 4V \frac{k}{b} \left(\epsilon^{-\frac{\pi^2}{b^2} xt} \cos \pi \frac{z}{b} + \epsilon^{-9 \frac{\pi^2}{b^2} xt} \cos 3\pi \frac{z}{b} + \dots \right),$$

a formula which has the same value at z and $(b-z)$. At any distance z and time t the flow of heat through unit area from the two boundary planes into the slab is the resultant of a number of sinusoidal heat waves $4k \frac{V}{b} \cos \pi \frac{z}{b}$; $4k \frac{V}{b} \cos 3\pi \frac{z}{b}$ etc. The higher their order n the more rapidly do these waves diminish, so that after a certain interval, t, only the longest wave is responsible for the heat transfer. The series occurring in the formula is once more a theta function:

$$C = 4k \frac{V}{b} \theta_2 \left(z/b, q \right), \text{ with } \epsilon^{-\frac{\pi^2}{b^2} xt} = q^{1/4}.$$

One Boundary Plane at a Fluctuating Temperature

When, in the "plate" method of measuring heat conductivity, one side of the slab is brought into close contact with a surface heated by alternating current, a steady state will be reached, in the course of time, such that the heat flowing into the plate through the hot wall during one half-cycle is equal to the heat flowing back during the next half, and it is of interest to ascertain how the heat flow varies with the thickness of the plate. On the assumption that the temperature at the surface $z=b$ changes according to the law $V\cos 2\pi ft$ or $V\cos pt$, f being the number of cycles per second, the temperature distribution is given by the relation (1, p. 212) in which $\mu = \sqrt{p/2x}$

$$v = \frac{V}{2}\left[\frac{\sin\mu(1+i)z}{\sin\mu(1+i)b}\,\epsilon^{-ipt} + \frac{\sin\mu\,(1-i)z}{\sin\mu\,(1-i)b}\,\epsilon^{+ipt}\right] +$$

$$\frac{2V}{b}\sum_{n=1}^{\infty}(-1)^n \sin n\frac{\pi}{b}z \;\frac{n^3\frac{\pi^3}{b^3}}{n^4\frac{\pi^4}{b^4}+\frac{p^2}{\varkappa^2}}\,\epsilon^{-n^2\frac{\pi^2}{b^2}\varkappa t} \;.$$

The flow of heat $-kdv/dz$ through unit area of the section at z and time t is therefore represented by the formula

$$C = -\frac{\mu kV}{2}\left[\frac{(1+i)\cos\mu\,(1+i)\,z}{\sin\mu\,(1+i)b}\,\epsilon^{-ipt} + \frac{(1-i)\cos\mu\,(1-i)z}{\sin\mu\,(1-i)\,b}\,\epsilon^{+ipt}\right] -$$

$$\frac{2kV}{b}\sum_{1}^{\infty}(-1)^n\cos n\frac{\pi}{b}z\;\frac{\frac{\pi^2}{b^4}}{\frac{\pi^2}{b^4}+\frac{4f^2}{n^4\varkappa^2}}\,\epsilon^{-n^2\frac{\pi^2}{b^2}\varkappa t} \;.$$

For the more common insulating materials $\varkappa$ varies from 5×10^{-4} to 5×10^{-3}, and f may vary from 10^{-5} cycles per sec. that is, about one cycle per day, to 100 or more cycles per sec. As b will usually amount to a few cm. at least the fraction in the second term is nearly unity for very slow changes; for frequencies above one cycle per sec. it is no more than a negligible fraction. The whole sum is thus smaller than $2k\frac{V}{b}\left(\theta(z/b,q)-1\right)$, and will vanish with time at about the same rate as the steady state is established in the case of the "plate" method. After about 10 min. only the first term contributes to the transfer of heat in the slab. In this stage the flow has become simply periodic so that the exchange of heat, H, per half-cycle is readily computed for the two boundary planes by integrating over the interval 0 to π/p and π/p to $2\,\pi/p$.

$$H = -\frac{i\mu kV}{2p}\left(\frac{(1+i)\cos\mu\,(1+i)z}{\sin\mu\,(1+i)\,b}\,\epsilon^{-ipt} - \frac{(1-i)\cos\mu\,(1-i)z}{\sin\mu\,(1-i)b}\,\epsilon^{+ipt}\right)_0^{\pi/p} .$$

This gives for the amount of heat entering or leaving each half-cycle at the side of the variable temperature the following expression

$$H_b = \pm\, 2\frac{\mu}{p}kV\,\frac{\sin h2\mu b - \sin 2\mu b}{\cos h2\mu b - \cos 2\,\mu b} \;.$$

As the thickness of the slab considered becomes greater, the exchange of heat tends towards the constant value $\pm\, k\; V\sqrt{1/\pi\,\varkappa f}$ in agreement with the formula given by the simpler theory developed for this special case (Table II).

TABLE II

CHANGE OF HEAT TRANSFER PER HALF-CYCLE WITH b

$2\mu b =$	$\pi/4$	$\pi/2$	$3\pi/4$	π	$5\pi/4$	$3\pi/2$	$7\pi/4$	2π
$H_b =$	0.260	0.690	0.75	0.92	1 0	1.02	1.01	1.00
$H_0 =$	−0.128	−0.134	−0.34	−0.398	−0.36	−0.13		−0.08

On the other hand the amount of heat flowing during each half-cycle into, or out from, the cold plane is given by the equation

$$H_o = \pm 2\frac{\mu}{p} k V \cdot \frac{\cos \mu b \sin h\mu b - \sin \mu b \cos h\mu b}{(\cos \mu b \sin h\mu b)^2 + (\sin \mu b \cos h\mu b)^2}$$

It has its highest value for small thicknesses. The difference, finally, between H_b and H_0 represents the capacity of storing heat during one half-cycle and releasing it during the next half.

Other periodic changes may be treated by combining a series of waves. The solution as given applies only to those cases in which the temperature of the boundary planes themselves undergoes fluctuations; when instead, the temperature of the surrounding air varies up and down, the contact or surface resistance which is offered to the heat flow by the surface of discontinuity must be taken into account. Its effect on heat transference through the slab will be most marked in the case of good conductors. The expression giving the heat flow also represents the distortion which an alternating electric current suffers in passing through a long transmission line or cable grounded at the farther end. It is necessary only to replace $\varkappa$ by $1/r_1c_1$, where r_1 is the resistance, c_1 the capacity per unit length.

Acknowledgment

The author is indebted to Mr. F. E. Lathe, who proposed the problem, for his interest and suggestions.

References

1. CARSLAW, H. S. Theory of the conduction of heat. 2nd ed., 1921.
2. GELIUS, S. Z. Ver. deut. Ing. 75: 369-370. 1931.
3. HAYASHI, KEIICHI, Tafeln der Bessel, Theta und anderer Funktionen. 1930.
4. HENCKY, K. Die Waermeverluste durch ebene Waende. 1921.

A CONVENIENT MECHANICAL MEANS OF WINDING QUARTZ SPIRALS[1]

By J. S. Tapp[2]

Abstract

A mechanical device has been constructed for the automatic winding of quartz spirals with any desired pitch. The resultant spirals are compact, accurate and uniform, and are produced with a minimum of personal attention. A study was made of the relationship between the sensitivity and the diameter of the fibre employed.

The machine itself is inexpensive and involves no complicated parts. About 50 successful spirals have been prepared in the manner recorded.

Preparation of Fibres

The most essential prerequisite was a uniform quartz fibre of the proper thickness. The quartz could best be drawn out by a falling weight acting through a system of strings and pulleys to give a horizontal pull. A foot-operated trigger held the weight in check and when released simultaneously removed the flame from the quartz. Fibres about 9 or 10 ft. long were successfully produced in this way and a little practice soon enabled the operator to predetermine, with considerable accuracy, the resultant diameter of the fibre by an adjustment of the amount of quartz heated. Since only 4 or 5 ft. of quartz fibre was necessary for a satisfactory spiral the unsuitable portions of the original 9 or 10 ft. could be discarded and in this way a sufficient length of the proper size could almost invariably be obtained.

The Winding

This operation, when performed by hand, required about 45 or 50 min. of very close attention and, even at best, the spiral obtained was unevenly spaced.

To dispense with this tedious process a machine was designed and constructed from "Erector" parts to perform the same task more easily and to produce uniform coils. The device automatically revolved the rod around which the fibre was being wound and at the same time advanced the rod horizontally so as to give the resultant spiral a uniform and adjustable pitch. Furthermore, there was maintained on the fibre a friction tension which could be conveniently altered during the course of the winding if desired.

The essential parts of the machine are shown in the accompanying sketch.

The power was supplied by a small six-volt d.c. high-speed electric motor, A, which revolved at about 3200 r.p.m. A pinion, B, on the motor shaft engaged the flat gear, C, which in turn through a pulley and belt caused the shaft, E, to revolve with a speed reduction of 9 to 1 relative to the motor. This shaft, by means of a worm gear, H, further reduced the speed (25 times) and conveyed the power to shaft L which, through a pinion and large flat gear, caused the chuck, R, to revolve at about 4 r.p.m. A 7 by $\frac{1}{4}$ in. carbon rod, S, was held

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Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada.

[2] *Demonstrator in Physical Chemistry, McGill University.*

in the chuck and kept in line by a free bearing, *T*, at the opposite end. Directly behind the large gear and turning with the chuck shaft was a three-inch flat steel disk, *F*, one face of which was covered with a sheet of thin cardboard. Against this disk was pressed a three-inch wheel with a narrow friction rim, *D*, mounted on a shaft at right angles to the one previously mentioned. By means of a pinion and crown gear (3-1) the movement of this shaft was communicated

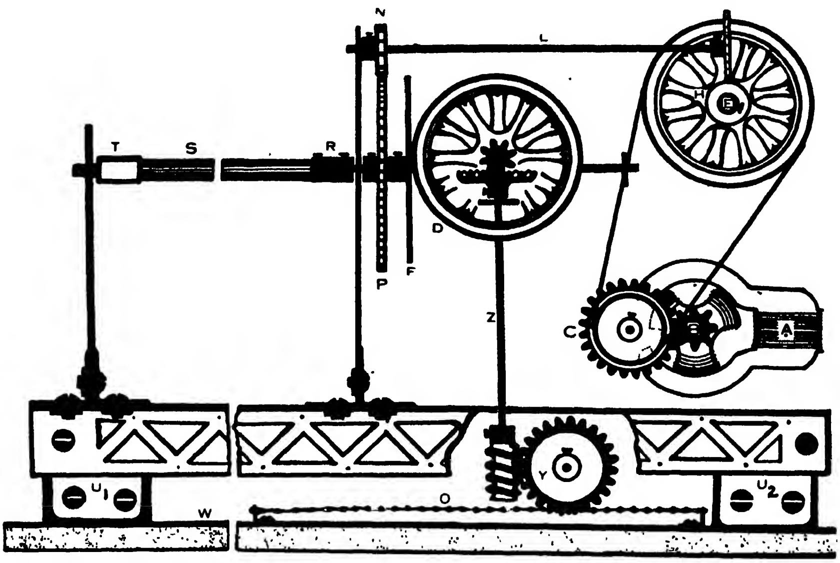

FIG. 1. *Diagram of apparatus built for the winding of the quartz spirals. (Length, about 18 in.; height, about 10 in.)*

to a vertical shaft, Z, extending down through the platform of the machine. This shaft had a worm gear attached at its lower end which meshed with a flat gear, *Y*, (25-1), mounted on a short shaft placed at right angles to the longitudinal axis of the machine. The lower part of this same gear meshed with a chain which was securely fastened level with the base and parallel with the longitudinal axis of the machine. As the chuck revolved, the system of gears just described caused the gear in contact with the chain to advance along its length at a speed determined by the radial position of the friction drive, and thereby carry along the whole machine at a uniform but adjustable rate of speed. (U_1 and U_2 are steel skids which support the machine and slide in a grooved track *W*.)

In Fig. 2 is shown the apparatus used for heating the fibre and applying tension. *A* is an air-blast gas burner, *B* the revolving carbon rod on which the spiral is wound, C_1 and C_2 are two plates with narrow vertical slits through which the fibre *H* passes. *D* is the friction plate bearing against a similar one beneath, and *E* is the tension adjusting screw. *N* is a pivot around which the upper part of the tension plate may be revolved to allow of convenient threading of the fibre. *R* is another pivot which permits the elevation or partial rotation

of the entire guide so as to assure correct alignment at all times. *Y* is a firm upright which is securely fastened to the wooden base and supports the guide and tension mechanism.

The heat required to soften the quartz thread was supplied by the air-blast gas burner mounted on a retort stand and held in position by a clamp. The exact position and intensity of this flame required a great deal of study and trial before it was correctly placed. In the first place it must be of sufficient intensity to soften, and yet not appreciably weaken, the fibre, for in the latter case the tension would cause the fibre to thin out and snap. A very hot flame directed at not quite the right angle was also useless, because once the quartz fibre touched the carbon rod no amount of heat would then cause it to become permanently bent. The best results were obtained when the peak of the blue inner cone of the flame just touched the fibre about 2 mm. in advance of the carbon rod, and when the axis of the flame, if produced, would pass down the centre of the incoming fibre. Many other arrangements were tried but none met with as good success as the one just described.

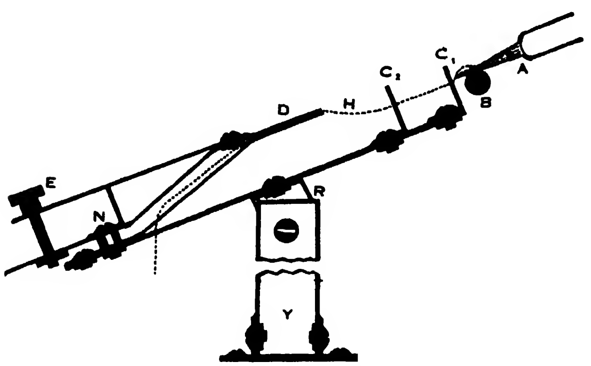

FIG. 2. *Diagram of the tension and guide device, mounted on the same base with the winding mechanism and placed at right angles to the plane of Fig. 1. in a position opposite the carbon rod (S).*

Since the amount of extension caused by any given force is directly proportional to the number of turns in the spiral and also to the diameter of the spiral, it would be advantageous to have as many turns as possible, and for the sake of compactness, as close together as possible. Increasing the diameter of the spiral proportionately reduces the number of turns that can be obtained from a specified length of quartz. In the author's opinion, the spiral with the small diameter and more numerous turns has an advantage in all cases, and certainly has in investigations carried out under high pressures where smallness of the container is a safety factor. The machine was capable of adjustment to give spirals with from 20 to 50 turns per inch as desired. The optimum was found to be about 42 or 43, any more than that resulted in occasional overlaps which were ruinous.

The spiral when finished was slipped off the carbon rod and neat rings formed at each end.

Calibration

The process of calibration consisted merely of suspending the spiral from some solid support and measuring with a cathetometer the length between the upper tip of the top ring and the lower tip of the bottom ring. This length

for any spiral was termed its "normal length". Small calibrated metal weights were then suspended from the bottom and the length measured as before.

Sensitivity

The weight in grams divided by the extension in mm. was termed the "sensitivity". The sensitivity, when multiplied by the fraction of a millimeter to which the cathetometer was capable of measuring accurately ($\frac{1}{20}$ mm. in this case) gave the "limit of detection" for the spiral in question.

The "maximum load" was that weight which caused an extension of two and one-half times the normal length.

Several experiments were carried out with spirals made of fibres of different diameters and it was found that, for a spiral of 80-90 turns, the relationship shown in Table I held approximately. All spirals were of the same diameter, about $\frac{3}{8}$ in.

TABLE I

RELATIONSHIP BETWEEN DIAMETER OF FIBRE AND SENSITIVITY OF SPIRAL (DIAMETER OF SPIRAL, ABOUT $\frac{3}{8}$ IN.)

Diameter of fibre, mm.	0.13–0.15	0.10 –0.11	0.09 –0.10	0.08 –0 09
Sensitivity, gm. per mm.	<0.03	0.011–0.018	0.0072–0 0080	0 0040–0 0060

References

1. McBain, J. W. and Baker, A. M. J. Am. Chem. Soc. 48: 690-695. 1926.
2. Pidgeon, L. M. and Maass, O. J. Am. Chem. Soc. 52: 1053-1068. 1930.

THE ELECTRICAL CONDUCTIVITY OF AQUEOUS SOLUTIONS OF HYDROGEN SULPHIDE AND THE STATE OF THE DISSOLVED GAS[1]

By R. H. Wright[2] and O. Maass[3]

Abstract

This paper is the last of three dealing with the equilibria between hydrogen sulphide and water, and is a continuation of a series of researches designed to investigate the equilibria existing in gaseous aqueous systems.

Electrical conductivities of aqueous solutions of hydrogen sulphide have been measured between 5° and 60°C. The results lead to the conclusion that hydrogen sulphide resembles certain other gaseous solutes and forms with water a complex which undergoes electrolytic dissociation. The constant k of the Ostwald dilution law therefore appears to be an apparent, rather than a real, dissociation constant.

The complete analysis of the equilibria involved and the evaluation of the constants must depend on accurate measurements at low concentrations, which are not yet completed.

Introduction

The state of hydrogen sulphide dissolved in water has hitherto been known only from the scattered observations of a number of observers. It has been the object of the work described in this and two preceding papers (10, 11) to secure an homogeneous body of information from which the nature of aqueous solutions of hydrogen sulphide could be deduced. To this end, the partition of hydrogen sulphide between the vapor and aqueous phases has been determined and the conductivities of the solutions have been measured. This paper is devoted to the latter of these measurements and the preliminary theoretical consideration of the problem as a whole.

The object of the partition experiments was to determine the solubility curves in such a way as to eliminate all sources of deviation from the ideal laws not arising in the liquid itself. For instance, an apparent departure from Henry's law (regarded as a particular case of the partition law) might easily be caused by failure of the hydrogen sulphide in the vapor to obey the simple gas law*. Any departure, therefore, from the partition law after allowing for these extraneous effects may tentatively be ascribed to conditions in the liquid such as association, dissociation, hydration, etc.

The electrolytic dissociation of hydrogen sulphide is known to take place in two steps but, as it has been well shown (2, 5, 6) that the constant of secondary dissociation is vanishingly small, in this work hydrogen sulphide will be treated as a monobasic acid.

*That allowance for this factor is indeed necessary is shown by the results in the previous paper (11), in Fig. 5 of which the solid lines show the actual variation of the concentration of the solution with concentration of hydrogen sulphide in the vapor, whereas the dotted lines represent the curves obtained when the vapor was treated as an ideal gas.

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Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Canada. Constructed from a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry, McGill University.

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The experimental data for the electrical conductivity submitted in this paper are the first that have been published having any claim to comprehensiveness or precision.

Determination of Electrical Conductivity

Experimental Method

Previous attempts to measure the electrical conductivity of hydrogen sulphide solutions have been hampered by experimental difficulties arising from polarization of unplatinized electrodes and occlusion of hydrogen sulphide by platinum black. Control and measurement of the concentration have also been difficult (1, 9).

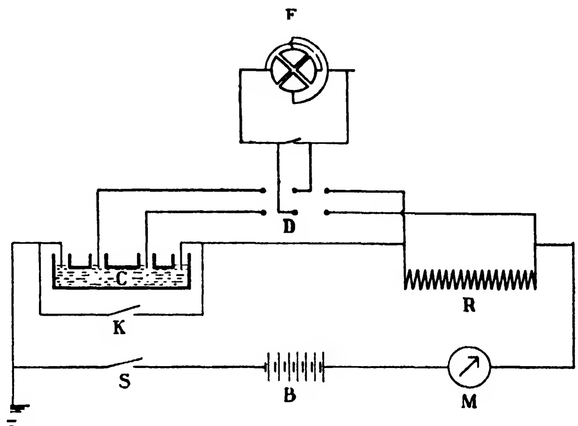

FIG. 1. *Diagram showing arrangement of apparatus for the electrical conductivity determinations.*

By adapting the sealed cell procedure described in the preceding paper (11), the latter of these difficulties has been overcome, and the problem of polarization has been avoided altogether by using the static method of conductivity measurement. This method is not new but it has lately been improved in these laboratories and elsewhere and seems quite satisfactory.

The principle of the method is very simple. If two resistances R_1 and R_2 be connected in series and a current passed through the circuit, then if E_1 is the potential across R_1, and E_2 the potential across R_2,

$$\frac{E_1}{E_2} = \frac{R_1}{R_2}.$$

Fig. 1 shows the application of this principle to the measurement of electrical conductivities. The conductivity cell, C, was connected in series with a resistance, R, milliammeter, M, battery of dry cells, B, and a switch, S. The cell was provided with two exploring or "secondary" electrodes which lay between the primary ones.

By means of the double pole, double throw switch, D, the secondary electrodes or, alternatively, the resistance, R, could be connected to the quadrants of a Dolezalek electrometer, E. Since the deflection of the electrometer was related to the potential difference of opposite pairs of quadrants, then, from the ratio of the deflections the ratio of potential fall between the exploring electrodes to the potential fall across the known resistance could be obtained. Since the current was the same through both, this ratio was also the ratio of the resistances. The absolute values of the potentials and of the primary current were within limits immaterial, so that the resistivity of the solution was found in terms of two electrometer deflections and a known resistance.

Since, however, the electrometer deflections were for various reasons not strictly proportional to the applied potentials, the electrometer was first calibrated by connecting a number of combinations of standard cells to the quadrants and plotting a curve of potential against deflection.

The usual precautions of insulation, shielding, etc., were taken in setting up the apparatus.*

The conductivity cell used is shown in Fig. 2. It was made of Pyrex glass and the electrodes were of thin platinum ribbon, unplatinized and adhering closely to solid glass supports as shown. The secondary electrodes, B and C, had a very much smaller surface than the primary and consisted simply of small platinum tips projecting into the side arms, the entire side arm constituting the exploring electrode.

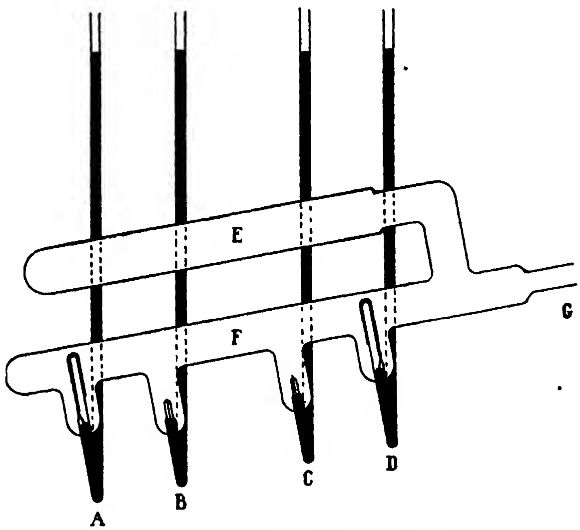

FIG. 2. *The conductivity cell.*

The cell was mounted on a mechanical rocker placed in a thermostat. The cell contents could be thoroughly stirred by rocking the cell back and forth between readings. The electrometer connections to the mercury wells were made through flexible wires and were not removed during the rocking.

A cell constant was determined using 0.02 N and 0.01 N KCl, the specific conductivities of which were taken as 0.002765 and 0.001413 respectively, at 25° C. (7, p. 213). The water correction was obtained from a blank run.

The procedure in filling the cell was similar to that described in the previous paper (11). The water was not, however, run directly into the conductivity cell from the weight pipette, but into a bulb temporarily sealed to the apparatus. In this bulb it was freed from dissolved air by freezing and melting *in vacuo* and was then distilled into E, Fig. 2. The hydrogen sulphide was purified and measured as previously described, and condensed in F with liquid air while the water in E was frozen with solid CO_2. The cell was then sealed off at G and the conductivity of the resulting solution measured at various temperatures. At the conclusion of the measurements the cell was opened and its volume found by filling with distilled water and weighing.

**It is usual to ground one pair of quadrants, but it was found in this work that better results were obtained by grounding a point in the primary circuit instead.*

Results

The concentration of the solution at each temperature was found as shown in the following specimen calculation. As the partition coefficient of hydrogen sulphide between liquid and vapor varies with the pressure, an approximate calculation was first made showing roughly the pressure in the cell. The appropriate value of the distribution coefficient was then selected from the data of the previous paper (11, Fig. 6).

Specimen Calculation

Temperature, 20° C.; wt. of H_2S, 0.7439 gm.; wt. of H_2O, 42.38 gm.; vol. of water, 42.49 cc.; approximate pressure, 2100 mm.; approx. wt. of H_2S in solution, 0.460 gm.; increment of volume due to dissolved gas (from mixture rule), 0.54 cc.; volume of solution, 43.03 cc.; volume of the cell, 113.08 cc.; volume of the vapor, 70.05 cc.; partition coefficient D (20° C. and 2100 mm.), 2.67.

$$\text{Weight of } H_2S \text{ in solution} = \frac{D \times (\text{vol. of soln.}) \times (\text{total wt. } H_2S)}{(\text{vol. of vapor}) + D \times (\text{vol. of soln.})}$$

$$= \frac{2.67 \times 43.03 \times 0.7439}{70.05 + 2.67 \times 43.03}$$

$$= 0.462 \text{ gm.}$$

$$\text{Molarity of solution,} \quad \frac{0.462 \times 1000}{34.08 \times 43.03}$$

$$= 0.0316.$$

From the scale readings, electrometer calibration curve and cell constant, the specific conductivity was obtained at a number of temperatures, and values at other temperatures were interpolated. To obtain the degree of dissociation, the limiting equivalent conductivity at infinite dilution was calculated from the data of Jellinek and Czerwinski (4) who measured the conductivity of NaSH solutions at 0°, 18° and 25°C. The ion conductances of Na^+ and H^+ were taken from the International Critical Tables (3). The limiting conductance of hydrogen sulphide at other temperatures was obtained by interpolation. These conductances are given in Table I.

TABLE I

LIMITING EQUIVALENT CONDUCTANCE OF HYDROGEN SULPHIDE

Temp., °C.	0	5	10	15	18	20	25	30	40	50	60
Λ	269	299	328	356	372	385	414	442	499	556	613

The constant, k, of the Ostwald dilution law (*i.e.*, the apparent dissociation constant) was calculated from the formula,

$$k = \frac{M\left(\frac{\Lambda_v}{\Lambda_o}\right)^2}{1 - \frac{\Lambda_v}{\Lambda_o}}$$

where, M is the molarity of the solution, Λ_v the equivalent conductivity at concentration M, and Λ_o the equivalent conductivity at infinite dilution.

The results are summarized for each temperature in Table II, and in Table III values of the apparent dissociation constant are collected and compared with those of other authors.

TABLE II

EXPERIMENTAL RESULTS

Total pressure	Partial pressure	Molarity	Specific cond. $\times 10^5$	$\frac{\Lambda_v}{\Lambda_o} \times 10^3$	$k \times 10^8$
At 5°C.					
607	601	0.141	2.44	0.578	4.72
903	897	.208	2.91	.479	4.77
1181	1175	.270	3.35	.415	4.65
At 10°C.					
684	675	.135	2.95	.665	5.99
1011	1002	.199	3 58	.547	5.98
1321	1312	.259	4 12	.485	6.09
1758	1749	.343	4.56	.406	5.64
At 15°C.					
758	745	.129	3.49	.759	7.45
1117	1104	.190	4 28	.631	7 59
1459	1446	.248	4.92	.557	7.70
1940	1927	.329	5 45	466	7.13
At 20°C.					
832	815	.124	4 04	.841	8.75
1224	1207	.182	4.98	.710	9.19
1611	1594	.240	5 72	.619	9 20
2125	2108	315	6 38	.526	8 71
At 25°C.					
907	883	.118	4 64	.948	10 6
1332	1308	.174	5.72	.794	11 0
1741	1717	228	6 55	.694	11 0
2308	2284	302	7.33	.586	10 4
At 30°C.					
982	950	.113	5 24	1 05	12.4
1436	1404	.167	6 48	.879	12.9
1879	1847	.218	7.41	.767	12.9
2480	2448	.289	8.31	.650	12.2
At 40°C.					
1132	1077	104	6.48	1.24	16.2
1639	1584	.153	8.02	1 05	16.9
2147	2092	.202	9.15	.910	16.7
2829	2774	.267	10.3	.771	15.9
At 50°C.					
1282	1190	.097	7.73	1.44	20.0
1849	1757	.142	9.53	1.21	20.8
2421	2329	.867	10 9	1.05	20.5
3163	3071	.245	12.3	.898	19.8
At 60°C.					
1444	1295	.091	9.00	1.62	23.8
2060	1911	.133	11 0	1.36	24.4
2689	2540	.175	12.6	1.17	24.2
3551	3405	.232	14 2	1.00	23.2

TABLE III

SUMMARY OF DILUTION LAW CONSTANTS

Temp., °C.	5	10	15	20	25	30	40	50	60
k, mean value $\times 10^8$	4.71	5.74	7.47	8.96	10.8	12.6	16.4	20.3	23.9

Values from the literature

Observer	Jellinek and Czerwinski (4)	Auerbach (1)	Walker and Cormack (9)
Temp., °C.	0	18	18
k	1×10^{-8}	9.1×10^{-8}	5.7×10^{-8}

Discussion

The most striking feature of the experimental results is the regular and marked increase in the dilution law constant, k, with rise in temperature. Applying the equation

$$\frac{d \ln K}{dT} = \frac{-q}{RT^2},$$

$+q$ will signify the heat evolved in the reaction

$$H_2S \longrightarrow H^+ + SH^- + q.$$

The ordinary method of integration can be applied using the formula,

$$\ln \frac{k_2}{k_1} = \frac{q}{R} - \left(\frac{1}{T_2} - \frac{1}{T_1}\right).$$

Taking values of k from Table III, it is found that integrating from 5° to 25°C. gives $+q = -6800$ cal., whereas integrating from 30° to 60°C. gives $+q = -4300$ cal.

So great a change in q can hardly be explained by Kirchoff's theorem in terms of changes in the heat capacities of the ions or the dissolved hydrogen sulphide, and another explanation must be sought.

By assuming that when hydrogen sulphide dissolves in water, the solvent and solute unite to form a complex which then undergoes electrolytic dissociation, the above result becomes intelligible. The quantity q will then depend on two independent quantities, Q_1 the heat evolved in forming the complex from hydrogen sulphide and water, and Q_2 the heat evolved in the ionization of this complex. If Q_2 has a negative value, *i.e.*, if the dissociation of the complex is attended by the absorption of heat (which is to be expected from the similarity of hydrogen sulphide and water, an increase in the extent of ionization would be expected with rise in temperature. This tendency would, however, be opposed by the decrease in the dielectric constant of water, and in any case the degree of ionization seldom varies extensively with temperature.

The observed increase in k may also be explained in another way. Supposing Q_1 to have a negative value, *i.e.*, that the H_2S-H_2O complex is endothermic, then a rise in temperature would favor its formation and would therefore produce an increase in the number of ions over and above any increase due to a change in the ionization constant of the complex.

Furthermore, it is evident that $-q$ is the net heat evolved when (a) the ions combine to form the complex and (b) when a part of the complex then dissociates into free hydrogen sulphide and water. Assuming an endothermic complex, the process (b) will be relatively more important at low than at high temperatures in so far as it contributes to the net heat evolution q. The marked decrease in q at higher temperatures is therefore readily explained by the hypothesis of an endothermic complex.

The system may now be provisionally formulated in the following way (secondary dissociation being neglected).

$$H_2S \text{ (in vapor)} \rightleftarrows H_2S \text{ (in solution)}.$$
$$H_2S \text{ (in soln.)} + nH_2O \rightleftarrows H_2S.nH_2O + Q_1.$$
$$H_2S.nH_2O \rightleftarrows H^+ + SH^-.nH_2O + Q_2.$$

Corresponding to each equilibrium there will be an equation:

$$\frac{[H_2S] \text{ soln.}}{[H_2S] \text{ vap.}} = h, \quad \frac{[H_2S]\,[H_2O]^n}{[H_2S.nH_2O]} = K_1, \quad \frac{[H_2S.nH_2O]}{[H^+]\;[SH^-\,nH_2O]} = K_2.$$

It is evident that the system is now analogous to the systems treated by Maass and Morgan (8), *viz.*, sulphur dioxide and water, carbon dioxide and water, and ammonia and water. There is however an important difference. In all the systems treated by these authors, the formula of the complex (H_2SO_3, H_2CO_3, and NH_4OH) could be inferred from the composition of the salts formed, whereas there is no such clue to the value to be assigned to n in the equations above.

Maass and Morgan found that measurements at high concentrations were necessary for the evaluation of K_1 and K_2 in the systems examined by them. From a consideration of the present problem, it appears that measurements with very low concentrations are also required when n has also to be determined. Since these have not yet been made, this paper can include only a qualitative consideration of the system.

The assumption of a complex such as has been described will also account, in a qualitative way, for the form of the vapor pressure curves of the preceding paper (11, Fig. 5). From the equation,

$$\frac{[H_2S]\,[H_2O]^n}{[H_2S.nH_2O]} = K_1$$

it is evident that as $[H_2S]$ increases (at constant temperature) $[H_2S.nH_2O]$ will also increase, but relatively more slowly owing to the diminution in $[H_2O]$. The net result of $[H_2S]$ increasing more rapidly than $[H_2S.nH_2O]$ will be a curvature in the solubility curves of the kind actually displayed by the isotherms of the previous paper (11).

References

1. Auerbach, F. Z. physik. Chem. 49: 217-223. 1904.
2. Auméras, M. Compt. rend. 186: 1724-1726. 1928.
3. International Critical Tables, v. 6, McGraw-Hill. 1929.

4. Jellinek, K. and Czerwinski, J. Z. physik. Chem. 102: 438-479. 1922.
5. Knox, J. Z. Elektrochem. 12: 477-481. 1906.
6. Knox, J. Trans. Faraday Soc. 4: 29-50. 1908.
7. Landolt-Börnstein. Physikalisch-chemische Tabellen. v. 2. Springer, Berlin. 1923.
8. Morgan, O. M. and Maass, O. Can. J. Research, 5: 162-199. 1931.
9. Walker, J. and Cormack, W. J. Chem. Soc. 77: 5-21. 1900.
10. Wright, R. H. and Maass, O. Can. J. Research, 5: 442-447. 1931.
11. Wright, R. H. and Maass, O. Can. J. Research, 6: 94-101. 1932.

AN EQUATION OF STATE FOR GASES AT LOW DENSITIES[1]

By D. LeB. Cooper[2] and O. Maass[3]

Abstract

An equation of state for gases at low densities is developed, using a new function for the change in viscosity with temperature, also developed herein.

The gas law equation takes the form

$$PV^2+a-RTV-\frac{RTb(1+KT_0)}{1+KT}=0$$

or $V(1+KT)(PV-RT)=\lambda T-a$ where a and b are constants corresponding to those of the Van der Waals' equation, and K is a constant derived from the proposed viscosity function which is, for carbon dioxide,

$$\eta=\sqrt{T}(1+KT)$$

where K is a constant and η is the viscosity at an absolute temperature T.

In the case of carbon dioxide the equation was found to follow density results with an accuracy of from 0.01% to within experimental limits, and the viscosity function was found to agree with Sutherland's (10) results between −78.5 and 20° C.

Comparisons with several other equations of state are made. These show that the new equation is probably more accurate than any other.

An expanded form of the new equation, namely:

$$\frac{M^1}{M_0}=1+\left(\frac{a-\dfrac{RTb_0}{1+KT}}{R^2T^2}\right)P+2\left(\frac{a-\dfrac{RTb_0}{1+KT}}{R^2T^2}\right)^2P^2\quad+\text{ etc.}$$

permits calculations of the slopes of isothermals for any temperature. Comparisons are made with experimental data.

The expanded form of the equation may be solved for K, giving the expression:

$$\frac{(\theta_1-\theta_2)\ (\theta_3+K)}{(\theta_1-\theta_3)\ (\theta_2+K)}=\frac{\lambda_1-\lambda_2}{\lambda_1-\lambda_3}$$

where $\theta=\dfrac{1}{T}$ and $\lambda=a-\dfrac{\xi}{\theta+K}$ and $\xi=Rb_0$, and since the equation enables the calculation of the molecular radius r, the viscosity may be calculated for any temperature and pressure over which the equation holds.

Introduction

The authors (5) have published the results of measurements of the density of carbon dioxide. The accuracy of the results was of the order of 0.01%, approximately 10 times that of previously existing data. The object of this communication is to apply these previously published results to the examination of some equations of state including a new one proposed herein.

The application of the new equation is restricted to gases at low densities. The restriction is intentional and succeeded a consideration of the possibilities of the usefulness of equations of state for deductions concerning molecular phenomena. These considerations are outlined below.

An infinitely dilute gas is usually considered "ideal". More correctly, it approaches the ideal as its density decreases. Increasingly large aberrations follow increasing density. Representation of these aberrations by correction

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terms applied to the ideal gas law equation presupposes at least partly analogous conditions between the actual and the ideal. Representation of high pressure data is complicated by the fact that neither is the term "molecular volume" unique, nor "mean free path" significant. As a consequence it appears to be a reasonable supposition that more pregnant deductions can be made by the use of low density data and equations holding in that region in which partially analogous conditions exist.

The proposed equation is developed by the use of a Van der Waals' (12) molecule. Possibly that developed by Lennard-Jones (8) has the greatest significance compatible with tractability, but with a restriction to the low density region and assumption that the effective molecular radius does not vary within the second order of small quantities, and furthermore that at low densities the attractive field is not appreciably affected by pressure, the use of a simpler Van der Waals' molecule is not only justified but preferable. This is more particularly true in the treatment of gases with large molecular fields and relatively soft molecules.

The degree of conformity of the equation based on these postulates determines whether or not the above assumptions are justified.

Mathematical Development of Equation

Maass and Mennie (9) have shown that the pressure of an ideal gas may be represented by the equation

$$\left(P+\frac{A}{V^2}\right)V=\left(1+\frac{2r}{l}\right)RT, \tag{1.0}$$

where r is the molecular radius, l the mean free path, A a Van der Waals' constant of attraction, and P, V, R and T have their usual significances. If, as postulated above, r and A remain effectively constant, the variation in the deviation (PV-RT) results from changes in l.

The variation in l may be represented as a function of the temperature.

Sutherland's (11) expression incorporated in equation (1.0) yielded the following:

$$PV^2-RTV+A-RTB\left(1+\frac{C}{T}\right)=0, \tag{1.1}$$

where

$$B=\frac{8\sqrt{2}\,\pi r^3 N}{1+\frac{C}{273}}, \tag{1.2}$$

or

$$PV^2-RTV+A-RTB^1=0. \tag{1.3}$$

Maass and Mennie (9) point out that this equation may be transformed into one identical with that of Van der Waals' by a first order approximation, and that in agreement with the fact that Sutherland's function fails at low temperatures, Equation (1.3) fails at temperatures below 0° C.

It was believed that an equation of this type was capable of further development provided a rigid function for variations in l with temperature could be found. The following is offered as a solution.

The mean free path l is related to the viscosity by an equation such that

$$\eta = K_1 mn\chi l, \tag{2.0}$$

where K_1 is an unknown constant. Or, more accurately, since η changes more rapidly than $\sqrt{T}$

$$\eta = K_1 mn\chi l f(T). \tag{2.1}$$

But

$$\chi \propto \sqrt{T},$$

therefore

$$\eta = K_1 mn\sqrt{T}\ l f(T), \tag{2.2}$$

and the viscosity at 0° C. is $l_0 = \dfrac{\psi}{4\sqrt{2}\ \pi r^2 n}$,

where ψ is some constant, and as $l = l_0$ when $\eta = \eta_0$ (assuming as before that the total change in η is caused by a change in l) therefore

$$\eta_0 = \frac{\psi K_2 mn\sqrt{T}f(T)}{4\sqrt{2}\ \pi r^2 n}, \tag{2.3}$$

whence

$$\eta = K_3\sqrt{T}f(T), \tag{2.4}$$

where

$$K_3 = \frac{\psi K_2 m}{4\sqrt{2}\ \pi r^2} = \text{const.}, \tag{2.4.1}$$

or

$$\frac{\eta}{\sqrt{T}} = K_3 f(T). \tag{2.5}$$

The data of Sutherland and Maass (10) was used to plot $\dfrac{\eta}{\sqrt{T}}$ against T for carbon dioxide and $f(T)$ was found to be a straight line, whence

$$\frac{\eta}{\sqrt{T}} = 1 + KT, \tag{2.6}$$

and we may write

$$l = l_0\frac{(1+KT)}{(1+KT_0)}, \tag{2.7}$$

whence substitution in (1.3) gives

$$\left(P + \frac{a}{V^2}\right) V = RT\left[1 + \frac{2r(1+KT_0)}{l_0(1+KT)}\right], \tag{3.0}$$

where a is a Van der Waals' constant of attraction differing numerically from A (Equation 1.3).

But

$$l_0 = \frac{\psi}{4\sqrt{2}\ \pi r^2 n},$$

whence

$$\left(P + \frac{a}{V^2}\right) V = RT\left[1 + \frac{8\sqrt{2}\ \pi r^3 n(1+KT_0)}{\psi V(1+KT)}\right], \tag{3.1}$$

or

$$PV^2 + a - RTV - \frac{RTb(1+KT_0)}{1+KT} = 0 \tag{3.2}$$

and

$$b = \frac{8\sqrt{2}\ \pi r^3 n}{\psi}.$$

Equation (3.2) may be written

$$V(1+KT)(PV-RT) = b_0 T - a + aKT, \tag{3.3}$$

where

$$b_0 = b(1+KT_0) = \text{const},$$

or

$$V(1+KT)(PV-RT) = \lambda T - a, \tag{3.4}$$

where

$$\lambda = b_0 + aK = \text{const}.$$

Equation (3.4) is the one used for comparisons. The constants K and λ may be evaluated easily and the equation tested for rigidity without difficulty, as inspection shows that the left hand term should vary linearly with T. That this is so is seen from Fig. 1, which shows that the equation holds over the measured range with the desired accuracy. The values of λ and a may be determined directly from the line of Fig. 1.

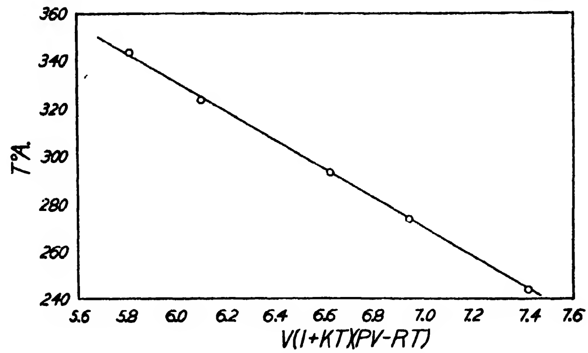


FIG. 1. *The calculated values of $V(1+KT)(PV-RT)$ plotted as a function of T in °A.*

The function may be evaluated as follows. We define a volume, V, such that V represents the actual volume occupied by a theoretical molecular weight of a gas at a corresponding T and P. V is then calculated from the published data from the equation,

$$V = \frac{M_0}{M^1}\frac{RT}{P}. \tag{A}$$

For purposes of correlation these values are shown in Table I.

TABLE I

APPARENT MOLECULAR WEIGHT AND VOLUME OF A GRAM MOLE OVER A TEMPERATURE RANGE

Temperature ° Abs.	Apparent molecular weight (M_1)	V (observed) litres
243.18	44.422	19.764
273.18	44.295	22.266
293.18	44.232	23.930
323.18	44.167	26 418
343.18	44.138	28 071

The values of RT may be calculated for each temperature and those of $1+KT$ from the viscosity line (Fig. 1) the constants of which are, for carbon dioxide, $1+4.094\times10^{-3}T$. The values of the left hand function lie on a straight line (Fig. 1) the equation for which is

$$\lambda T - a = 1.6167\times10^{-2} - 11.34.$$

The numerical values of the results are shown in Table II, and the exactitude of the equation is demonstrated further by a direct calculation of V shown in comparison with those calculated from several other equations (Table II).

TABLE II

COMPARISON OF RESULTS, *V obs.* AND *V calc.* AT VARIOUS TEMPERATURES; PRESSURE CONSTANT AND EQUAL TO ONE ATMOSPHERE

Temperature ° Abs.	*V obs.*, litres	V_1*, litres	V_2, litres	V_3, litres
243.18	19.764	19.764	19.772	19.952
273.18	22.266	22.266	22.265	22.414
293.18	23.930	23.930	23.930	24.055
323.18	26.418	26.418	26.418	26.517
343.18	28.071	28.071	28.069	28.157

* *V_1 = new equation; V_2 = Maass-Mennie equation; V_3 = gas law.*

The equation for carbon dioxide is

$$V(1+4.0983\times10^{-3}T)(PV-RT)=1.616\times10^{-2}T-11.34, \tag{3.5}$$

or, written in a form to show minor constants,

$$PV^2+11.34-RTV-RT\frac{0.3596(1+4.0983\times10^{-3}T_0)}{1+4.0983\times10^{-3}T}=0 \tag{3.6}$$

Discussion of the Equation

The equation may be written in the form of an isothermal by substitution of equation (A) and expansion by the binomial theorem. Then,

$$\frac{M^1}{M_0}=1+\left(\frac{a-\frac{RTb_0}{1+KT}}{R^2T^2}\right)P+2\left(\frac{a-\frac{RTb_0}{1+KT}}{R^2T^2}\right)^2P^2+\ldots, \tag{4.0}$$

where $b_0=b(1+KT_0)$.

The slopes of the isothermals may be calculated from the coefficient of P. These are shown compared with those determined experimentally in Table III.

TABLE III
SLOPES OF LOW PRESSURE ISOTHERMALS

Temperature, ° Abs.	u_1 (observed)	u_2 (calculated)
243.18	0.418	0.413
273.18	0.291	0.288
293.18	0.228	0.229
323.18	0.163	0.164
343.18	0.134	0.135

A variation of 0.01% in the highest molecular weight on any isothermal results in a change of slope of 18%. The calculated values are well within the limits of error. The coefficient of P^2 allows for curvature of the isothermals. It becomes effective at five atmospheres to the extent of 0.01%.

Following are similar expansions of the equations of Maass and Mennie, and Van der Waals, shown for comparison:

Maass and Mennie,

$$\frac{M^1}{M_0}=1+\left(\frac{A-RTB^1}{R^2T^2}\right)P+2\left(\frac{A-RTB^1}{R^2T^2}\right)^2P^2+\ldots\text{ etc.}$$

Van der Waals',

$$\frac{M^1}{M_0}=1-\frac{\alpha-RT\beta}{R^2T^2}P+\ldots\ldots\ldots\text{ etc.}$$

A second method of treatment of the expanded form (4.0) will be discussed later.

Comparison of Equations

Table II and succeeding tables serve to compare the several equations.

TABLE IV
COMPARISON OF RESULTS FROM BRIDGEMAN'S EQUATION FOR AN ISOSTERE AT $V=23.930$ l.

Temp.	*P obs.*	*P calc.*
243.18	0.82730	0.82664
273.18	0.93098	0.93046
293.18	1.0000	0.99970
323.18	1.1035	1.1032
343.18	1.1724	1.1723

Table II shows the calculated volume V compared with those determined experimentally. The values of the Maass-Mennie equation were calculated using their method with constants derived from the published results of the authors. The discrepancy at −30° C. is well outside the limits of error.

Table IV shows comparisons with Bridgeman's equation for carbon dioxide, calculated in the form of an isobar. At the lower tem-

peratures the deviations are greater than experimental errors.

TABLE V

COMPARISON OF THE OBSERVED PRESSURES WITH THOSE CALCULATED FROM VAN DER WAALS' EQUATION USING DIFFERENT VALUES FOR THE CONSTANTS α AND β

(1)	(2)	(3)	(4)	(5)	(6)
		α			
		4.208	7.06	7.56	4.208
Temp., ° Abs.	*P. obs.*	β			
		0.050	0.166	0.188	0.0712
		P. calc.			
243.18	1.0000	1.0013	1.0000	1.0003	1.0024
273.18	1.0000	1.0005	1.0000	0.9999	1.0014
293.18	(1.0000)	(1.0000)	(1.0000)	(1.0000)	1.0009
323.18	1.0000	0.9996	1.0000	1.0001	1.0004
343.18	1.0000	0.9995	1.0000	1.0003	1.0004

Table V requires further discussion. Attempts have been made to calculate the constancy of Van der Waals' a and b, written here, α and β. Algebraically they are unique, and hitherto it has been impossible to detect differences between calculated and experimental values even with considerable differences in the values of the constants used. The more accurate results show that a 7% variation in α causes a noticeable difference at the extreme temperatures even when the volumes are calculated using a corresponding β.

The values of α and β were obtained by a simultaneous solution of Van der Waals' equation using the authors' results: $\alpha = 7.06$; $\beta = 0.166$. Pressures calculated from these values follow the experimental results (column 4). Those calculated (column 6) from the best representative values of the constants based on the results of Amagat (1, p.109) and Andrews (2) and quoted by Jellinek (7, p. 632) show discrepancies varying from 0.2% at low temperatures to 0.03% at the highest. The figures of column 3 were calculated by substitution of the best representative value of α and calculation with a corresponding β.

The results in Table V show that a change in the constants of Van der Waals' equation makes it inapplicable to calculations of the highest accuracy.

Further comparison of Equations (1.3), (3.2) and Van der Waals' is made possible by the fact that the equations are similar when

$$RTB^1 = PV\beta - \frac{\alpha\beta}{V} = \frac{Rb_0T}{1+KT},$$

and, with the first order approximation that $PV = RT$,

$$RTB^1 = RT\beta - \frac{\alpha\beta}{V} = \frac{Rb_0T}{1+KT}.$$

The term $\frac{\alpha\beta}{V}$ affects the results to $\frac{1}{6000}$ and neglecting this,

$$B^1 = \beta = \frac{b_0}{1+KT},$$

or at 0° C.

$$B^1 = \beta = b.$$

TABLE VI
COMPARISON OF V *obs.* AND V *calc.* AT HIGHER PRESSURES

Pressure, obs.	Pressure, (Van der Waals' equation)	Pressure (new equation)
12.01	12.12	12.012
13.22	13.35	13.197
14.68	14.86	14.663
20.01	20.74	19.945
34.49	30.86	33.297

This indicates that at all temperatures other than 0° C. the proposed equation demands a variable, and the others a constant correction factor.

Table VI shows the range over which the new equation holds compared to that of Van der Waals'. The values for the latter were calculated by use of constants obtained from data of the authors. The constants for the proposed equation were obtained, as explained, by a process yielding unique values, for if the value of K be changed 100%, *i.e.*, be placed equal to zero, calculated and experimental values do not agree as is shown in Table VII.

TABLE VII
SHOWING EFFECT OF A CHANGE IN K ON AGREEMENT BETWEEN OBSERVED AND CALCULATED PRESSURES

Temp., ° Abs.	Obs. press.	Calculated pressure	
		$K=0$	$K=4.50$
243.18	1.0000	0.99884	1.0000
343.18	1.0000	1.00150	0.9999

The forms of the equations allow another differentiation. The new equation permits calculations of the slopes of the isothermals as shown above, but Van der Waals' equation has no like application due to the substitution of PV by RT.

Comparison with the equation of Bridgeman (4) is more difficult on account of the relatively large number of semi-empirical constants therein. Table IV indicates that while somewhat similar in form, Bridgeman's equation fails at low temperatures and pressures. At higher temperatures and pressures the agreement is satisfactory.

A more apparent difference between the three equations first mentioned may be detected by writing them in the form of isosteres, whence, for Maass and Mennie, and Van der Waals' we have

$$P=BT-A,$$

and for the recently proposed equation,

$$P=B_1T+\frac{C_1}{1+KT}-a.$$

The first two demand straight isosteres, the latter allows for a curvature. In this respect the new equation probably represents the facts to a greater degree of exactness since isosteres are known to possess a curvature at higher pressures. No curvature was detected in the isothermals, hence no value of C_1 can be calculated. An upper limit was found to be of the order of 1×10^{-4}.

Further Applications of the Proposed Equation

Among others the equation lends itself to two further applications, namely, the calculation of the molecular radius, r, and the viscosity constant K, as defined by Equation (2.6). A knowledge of r permits the calculation of the

viscosity of the gas under examination at 0° C., which value, substituted in Equation (2.6), permits, with a knowledge of K (also calculated from gas law relationships by use of the new equation), a calculation of the viscosity at any temperature over which the equation holds. In so far as the second application is concerned this is the first time that the calculation of viscosities at different temperatures has been possible from PVT results only.

The Molecular Radius

The calculation of the molecular radius may be carried out as follows:
From equation (3.1)

$$PV^2+a-RTV-\frac{RTb(1+KT_0)}{\psi(1+KT)}=0$$

where
$$b=8\sqrt{2}\,\pi r^3 n,$$

therefore $b=6\sqrt{2}\,V_0$, where V_0 is the total volume of the molecules; ψ is a correction factor. Using Jean's calculation of ψ, we have

$$PV^2+a-RTV-\frac{6\sqrt{2}\,V_0(1+KT_0)}{1.382(1+KT)}=0,$$

from which $r=2.28\times10^{-8}$ cm., the calculated viscosity at 0° C. of 0.0001353 differing from the experimental value of 0.0001354 by 0.15%.

The Viscosity Constant

The second application, namely, the calculation of the viscosity constant K from PVT data, may be carried out as follows.

From equation (4.0) we have

$$\frac{M^1}{M_0}=1+\left(\frac{a-\dfrac{RTb_0}{1+KT}}{R^2T^2}\right)P, \tag{6.0}$$

but
$$M^1=M_0+uP,$$

where u is a constant

therefore
$$\frac{M^1}{M_0}=1+\frac{u}{M_0}P, \tag{6.1}$$

and equating equal coefficients,

$$\lambda=a-\frac{\xi T}{1+KT}, \tag{6.2}$$

where $\lambda=\frac{u}{M_0}R^2T^2$, and $\xi=Rb_0$.

To solve for K, we define some quantity θ such that $\theta=\frac{1}{T}$

then
$$\lambda=a-\frac{\xi}{\theta+K}, \tag{6.3}$$

and eliminating a
$$\frac{\xi(\theta_2-\theta_1)}{(\theta_1+K)(\theta_2+K)}=\lambda_1-\lambda_2. \tag{6.4}$$

Similarly
$$\frac{\xi(\theta_3-\theta_1)}{(\theta_1+K)(\theta_3+K)}=\lambda_1-\lambda_3, \tag{6.4.1}$$

and by division
$$\frac{(\theta_1-\theta_2)(\theta_3+K)}{(\theta_1-\theta_3)(\theta_2+K)}=\frac{\lambda_1-\lambda_2}{\lambda_1-\lambda_3}, \tag{6.5}$$

and since λ may be evaluated for each experimental isothermal, K may be calculated directly.

The nature of the equation is such that K may be evaluated to one significant figure only, its value being 5×10^{-3}. Using this value for a calculation of b we have, $b = 0.412$, from which the viscosity at 0° C. is 0.000131, compared with the measured value of 0.0001354, and using the value of K above, b at −60° C. is 0.000116 against a measured value of 0.0001160 for carbon dioxide. Thus without the use of any results other than those used to determine the slopes of the isothermals, viscosities may be calculated over a long temperature range with an accuracy of about 2%. The nature of the viscosity temperature function has, however, to be assumed.

References

1. AMAGAT, E. H. Ann. chim. phys. 29: 68-136. 1893.
2. ANDREWS, T. Phil. Trans. 159: 575-590. 1869.
3. ANDREWS, T. Phil. Trans. 167: 421. 1877.
4. BRIDGEMAN, O. C. J. Am. Chem. Soc. 49: 1130-1138. 1927.
5. COOPER, D. LEB. and MAASS, O. Can. J. Research, 4: 283-298. 1931.
6. JEANS, J. H. The dynamical theory of gases, 4th ed. 1925.
7. JELLINEK, K. Lehr. d. phy. Chem. 1928.
8. LENNARD-JONES, J. E. Proc. Cambridge Phil. Soc. 22: 105. 1924.
9. MAASS, O. and MENNIE, J. H. Proc. Roy. Soc. A. 110: 198-232. 1926.
10. SUTHERLAND, B. P. and MAASS, O. Can. J. Research, 5: 428-443. 1932.
11. SUTHERLAND, W. Phil. Mag. 36: 507-531. 1893.
12. VAN DER WAALS. Die Kontinuitat des gasformigen und flussigen Zustandes. Leipzig. 1881.

THE CONDENSATION OF CERTAIN γ-KETONIC ESTERS WITH AROMATIC ALDEHYDES[1]

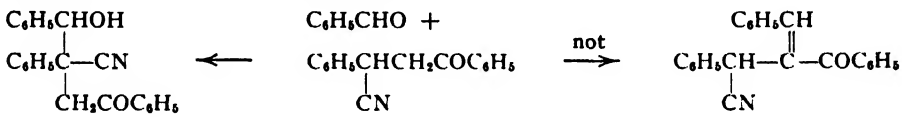
By C. F. H. Allen[2] and G. F. Frame[3]

Abstract

The condensation of methyl and ethyl α-phenyl-β-(para-substituted)benzoyl propionates with benzaldehyde and piperonal in the presence of sodium methylate, followed by acidification, has been found to produce cyclic compounds; the latter are shown to be lactols, six of which are described. The spontaneous ring closure is probably due to the highly branched chain. A mechanism for the reaction is proposed.

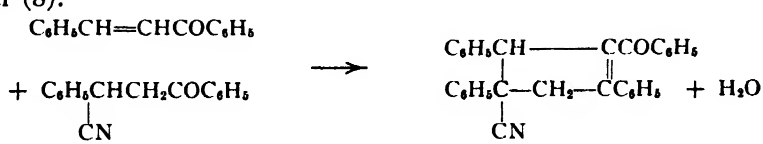
On oxidation with chromic acid, α-diketones are formed; the latter contain the aryl group introduced as aldehyde. The lactols resemble triphenylcarbinol in several respects, forming a chloride, methyl ether, and acetate on appropriate treatment, all of which, on hydrolysis, regenerate the lactol. They also give colored solutions with concentrated sulphuric acid, from which the starting material is recovered on addition to water. From this work it is evident that in arylated γ-ketonic esters the hydrogen atom alpha to the ketone carbonyl group is more active in alkaline aldol condensations than the hydrogen in the alpha position to the carbalkoxy carbonyl group; the observation of others, that the conjugated system $>C{=}C{-}C({=}O){-}OR$ is more stable than $>C{=}C{-}C({=}O){-}R$, has been confirmed.

In a previous investigation (12*) it was found that when benzaldehyde reacted with α-phenyl-β-benzoylpropionitrile (I) in an alkaline medium, the hydrogen in the position alpha to the cyano group was involved:



II I

In the preparation of α-phenyl-β-benzoylpropionitrile by treatment of benzalacetophenone with an aqueous solution of potassium cyanide, a high melting insoluble solid is the principal product unless the hydrogen ion concentration is carefully adjusted; this solid has been shown to be III, formed by addition of the nitrile first formed to a second molecule of unsaturated ketone, and loss of water (8).



III

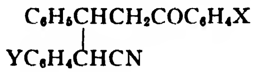
[1] *Manuscript received May 20, 1932.*
Contribution from the Department of Chemistry, McGill University, Montreal, Canada, with financial assistance from the National Research Council of Canada.

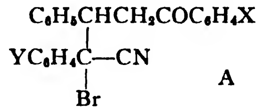
[2] *Assistant Professor of Chemistry, McGill University.*

[3] *Graduate student, McGill University, and holder, at the time, of a studentship and fellowship under the National Research Council of Canada.*

**In the first sentence, 3rd line, page 1353, the word "nitrile" is ambiguous; it should read α-phenyl-β-benzoylpropionitrile.*

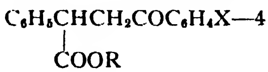
Thus the hydrogen atom alpha to the cyanogen radical is more active than the one alpha to the carbonyl group. Further examples are found in certain δ-ketonic nitriles (1, 12) that on bromination give almost entirely bromine substitution products of type A.





A

On account of these results it seemed desirable to investigate the ester (IV) corresponding to the nitrile (I) and determine which was the more active hydrogen. It was known that the acid (V) on bromination gave the mono-bromo-substitution product (VI), indicating that the hydrogen alpha to the ketonic carbonyl group was more reactive (14).



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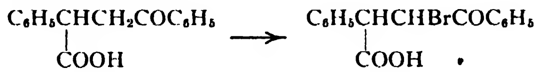
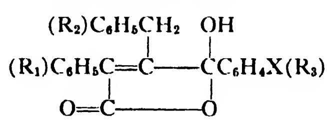


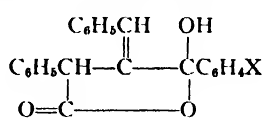
V VI

In this paper there will be described the products obtained from several esters (where X = Cl, Br, OCH_3) and benzaldehyde and piperonal. The aryl groups are "tagged" so that they can be followed through in reactions used to prove structure. Since a chlorinated lactone was first prepared this will be used in illustrating the reactions.

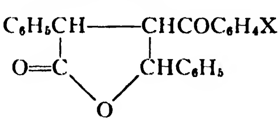
When methyl α-phenyl-β-(*p*-chloro-benzoyl) propionate and benzaldehyde are treated with sodium methylate in absolute methanol, a lactone, $C_{23}H_{17}O_3Cl$, is formed. As the same lactone results when the ethyl ester and ethyl alcohol are used, the carbalkoxy group must be involved in the formation of the lactone ring. It is possible to write eight possible isomeric structures, of which one (XIV) is a beta lactone; the latter is excluded because the product shows none of the characteristic properties of this type of cyclic compounds.



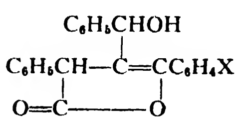
VII



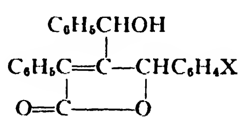
VIII



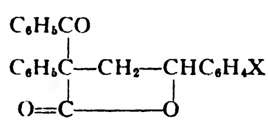
IX



X



XI



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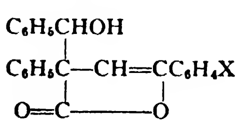
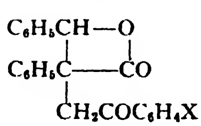


XIII



XIV

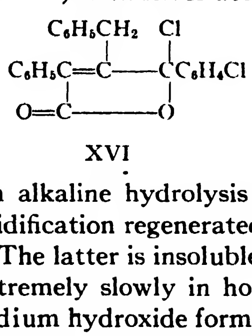
$C_6H_5CH_2COCOC_6H_4X$

XV

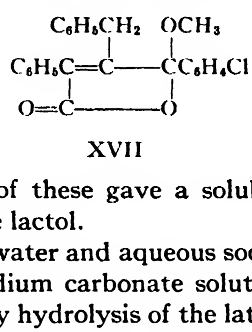
Any of the γ-lactones might equally well be formed; to distinguish between them has required a careful study of the reactions.

Potassium permanganate was rapidly reduced but the oxidation could not be controlled and a mixture of benzoic and *p*-chlorbenzoic acids was formed. Chromic acid was the most useful oxidizing agent. In this case benzyl *p*-chlorophenyl diketone (XV) resulted, which at once excluded substances having structures XII-XIV. By substituting piperonal for benzaldehyde and oxidizing the resulting lactone, piperonyl *p*-chlorophenyl diketone was obtained, showing that it was the aryl group introduced as aldehyde that appeared in the diketone. This would exclude a formula like VIII. Further, the substance was insensitive to ozone, whereas benzaldehyde would have been easily formed from VIII. Finally, the isomeric piperonal lactone (Formula VII, R_1 = 3,4-methylenedioxyphenyl, R_2 = phenyl, R_3 = *p*-chlorophenyl) has been made in this laboratory (21), and on oxidation found to form benzyl *p*-chlorophenyl diketone (XV). This evidence, taken altogether, excludes all the formulas except VII.

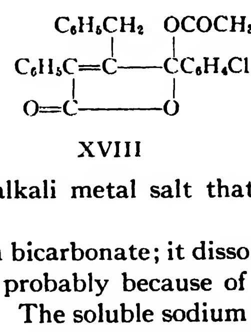
In the Grignard machine (15) the lactone shows one active hydrogen and two additions, indicating the presence of an hydroxyl group, but no esters were formed in the usual ways, as would be expected of substances X and XI; a lactone like IX would probably have no active hydrogen and should add three molecules of RMgX. On treatment of the lactone with acetyl chloride a chloride was obtained instead of an acetate, a characteristic property of tertiary alcohols. Thionyl chloride formed the same halide. This chloride (XVI) exhibited many of the characteristic properties of triphenylchlormethane. It formed a methyl ether (XVII) with absolute methanol, and an acetate (XVIII) with silver acetate. The lactone is thus a lactol.



XVI



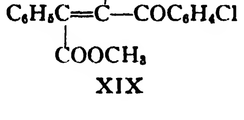
XVII



XVIII

On alkaline hydrolysis all of these gave a soluble alkali metal salt that on acidification regenerated the lactol.

The latter is insoluble in water and aqueous sodium bicarbonate; it dissolves extremely slowly in hot sodium carbonate solution, probably because of the sodium hydroxide formed by hydrolysis of the latter. The soluble sodium salt is readily converted into a silver salt in the usual way, and the latter gives a methyl ester (XIX) when boiled with methyl iodide. The lactol is re-formed by alkaline hydrolysis of the ester and acidification. The only evidence for the presence of the tautomeric open chain form (XX) is the formation of an oxime (XXI).



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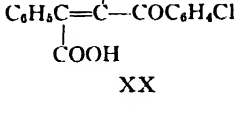

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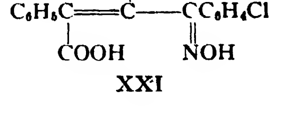
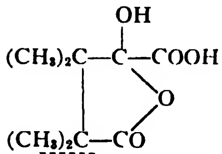
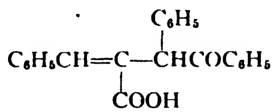
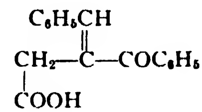


XXI

A detailed study of certain lactols has been made in connection with keto-lactol tautomerism (3, 6, 17, 22, 23, 24) as well as a few instances in suitably constituted substances having carboxyl and aldehyde groups in the required positions in the molecule (4, 9, 10, 19, 20). In nearly all the cases, typical reactions indicating both open chain and cyclic structure were observed, but with few exceptions, derivatives of the cyclic forms could only be obtained by drastic treatment (*e.g.*, with acetyl chloride or acetic anhydride). A stable lactol was formed only with substances having a very highly branched chain (XXII) (23).

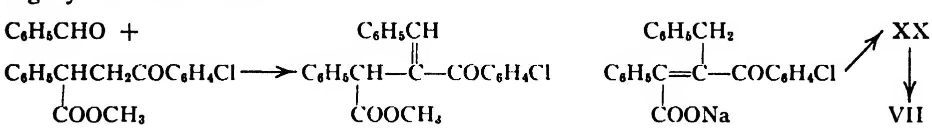

XXII


XXIII


XXIV

The most probable reason for the relative ease with which the lactols described in this paper are formed, simply acidification of the alkali salt, is doubtless the presence of the very highly branched chain, since very closely related substances (XXIII, XXIV) have stable open chain forms, and only give derivatives of a cyclic structure on treatment with acetic anhydride (5, 7).

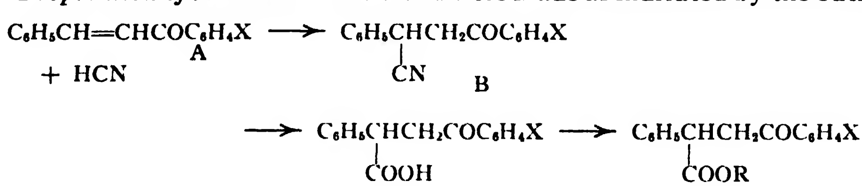
The mechanism of the reaction forming the lactol is probably as follows: the ester first adds to the aldehyde in the ordinary way, followed by elimination of water. The point at which the H and double bond shift is uncertain but since Thiele (25) has shown that β, γ -esters of this type (XXV) isomerize in alkaline media to give α, β-esters the change is exactly what would be expected. The water eliminated in the first step in the alkaline solution hydrolyzes the ester to an alkali salt; on acidifying the solution the free acid at once cyclicizes to the lactol, the tendency to ring formation being greatly increased by the highly branched chain.


XXV

The condensation was not brought about by piperidine or diethylamine.

Experimental

A. Preparation of the esters. The esters were made as indicated by the outline:


C D

(*1*) *The unsaturated ketones* (*A*). Benzalacetophenone was prepared according to the directions given in Organic Syntheses (13). Benzal *p*-chloro-, *p*-bromo-, and *p*-methoxyacetophenone were made by the following modification: to a mixture of 173 gm. of benzaldehyde, 183 gm. of *p*-chloroacetophenone, and 360 cc. of alcohol was added 78 cc. of 10% sodium hydroxide solution, and the whole cooled under the tap. The mixture became semi-solid in a few seconds. The whole was shaken frequently for 1.5 hr., then the solid filtered and purified. The yield was 267 gm. (93%) and the melting point 96° C. The *p*-bromo and *p*-methoxy homologues melted at 97° and 104° C. respectively.

(*2*) *The nitriles; addition of HCN* (*B*). α-Phenyl-β-benzoyl-propionitrile was prepared by the method given in Organic Syntheses (2). For the homologues it was found essential to operate for a longer time and at higher temperatures, and to use fresh alcohol in each run; *e.g.*, to 200 gm. of benzal-*p*-chloro-acetophenone, 2845 cc. of 95% ethyl alcohol and 87 gm. of glacial acetic acid was added 128 gm. of potassium cyanide in 354 cc. of water in 15 min., and the whole stirred at 35° C. for eight hours. After standing in the ice box, 193 gm. (87%) of α-phenyl-β-(*p*-chlorobenzoyl)propionitrile, m.p. 122° C., was obtained. The *p*-bromonitrile mixture was stirred for six hours and left in the ice box for two days. In preparing the *p*-methoxynitrile the temperature had to be kept at 50-55° C.; both were recrystallized from methyl alcohol.

TABLE I
YIELD, PROPERTIES AND ANALYSES OF THE NITRILES

Nitrile	Yield %	M.p. ° C.	Crystal form	Formula	Calcd. %	Found %
p-Bromo-	84	124	rectangular plates	$C_{16}H_{12}ONBr$	Br, 25.5	Br, 25.3
p-Methoxy-	65	62	long needles	$C_{17}H_{15}O_2N$	C, 77.0; H, 5.7	C, 76.8; H, 5.8

(*3*) *The esters* (*D*). Some of the esters were obtained by hydrolyzing the nitrile to the acid and esterifying the latter (18), and the others directly, by saturating absolute methyl alcoholic solutions of the nitriles with hydrogen chloride (16), using whichever was found to give the better yield (*e.g.*, with the *p*-chloro derivative, the first method gave a yield of 84% and the second 75%).

TABLE II
PROPERTIES AND ANALYSES OF THE ESTERS

Ester	M.p. ° C.	Crystal form	Formula	Calcd. %	Found %
Ethyl, *p*-chloro-	63	fine prisms	$C_{18}H_{17}O_3Cl$	Cl, 11.2	Cl, 11.5
Methyl, *p*-bromo-	129	rhombic plates	$C_{17}H_{15}O_3Br$	Br, 23.0	Br, 22.9
Methyl, *p*-methoxy-	97	dense prisms	$C_{18}H_{18}O_4$	C, 72.5; H, 6.0	C, 72.3; H, 5.9

The p-methoxyacid (C) though isolated (cf., first method) was not analyzed; by titration with standard alkali, a molecular weight of 275 was found (calcd. =284). It formed small scales from methyl alcohol.

B. Condensation of the esters with the aldehydes. A solution of sodium methylate prepared from 12.6 gm. of sodium and 212 cc. of absolute methyl alcohol was added to a mixture of 106 gm. of benzaldehyde, 212 cc. of absolute methyl alcohol, and 160 gm. of methyl α-phenyl-β-(*p*-chlorobenzoyl)propionitrile, and the whole refluxed for two hours. It was then acidified with acetic acid and the alcohol and unused aldehyde steam distilled. The residual organic product was taken up in ether, the latter dried with calcium chloride, and the solvent removed. The solid left was dissolved in the smallest possible amount of boiling methyl alcohol and deposited as fine prisms on cooling; the analytical sample was recrystallized from an ether-petroleum ether mixture. It is insoluble in petroleum ether, moderately soluble in alcohol and benzene, and very soluble in ether and acetone.

The same lactol resulted when ethyl alcohol and the ethyl ester were substituted in the above, and also when the reaction was carried out in alcohol that had been distilled from magnesium methylate. The homologues were prepared by essentially the same procedure, except that the ethereal extract was shaken with a saturated solution of sodium bisulphite when piperonal was used. Their properties are collected in Table III.

TABLE III
PROPERTIES OF THE LACTOLS

Lactol								Calcd., %			Found, %		
No.	R_1	R_2	R_3	Crystal form	M.p. °C.	Yield %	Formula	Hlg.	C	H	Hlg.	C	H
A	φ[c]	φ	4-Clφ	prisms[a]	134	77,82[e]	$C_{23}H_{17}O_3Cl$	9.4	73.4	4.5	9.3	73.5	4.5
B	φ	pip-[d]	4-Clφ	needles[b]	174	73	$C_{24}H_{17}O_5Cl$	8.4			8.3		
C	φ	φ	4 Brφ	prisms[a]	155	68	$C_{23}H_{17}O_3Br$	18.9			18.7		
D	φ	pip-	4-Brφ	needles[b]	171	88	$C_{24}H_{17}O_5Br$	17.2			17.6		
E	φ	φ	4-CH_3Oφ	plates[a]	119	92	$C_{24}H_{20}O_4$		77.4	5.4		77.7	5.4
F	φ	pip-	4-CH_3Oφ	needles[a]	162	92	$C_{25}H_{20}O_6$		72.1	4.8		72.2	4.7

[a] *From methyl alcohol;* [b] *from benzene;* [c] *φ = C_6H_5;* [d] *pip- = piperonyl;* [e] *from ethyl ester.*

C. General properties of the lactols (VII). The lactols are insoluble in sodium bicarbonate solution, but dissolve rapidly in cold, dilute sodium hydroxide, and are reprecipitated on acidification. They are recovered unchanged after solution in concentrated sulphuric acid. In the Grignard machine (15) they reacted with three moles of the reagent and evolved one mole of gas, indicating one active hydrogen and addition of two groups. They did not form esters (benzoates, *p*-nitro- and 3,5-dinitrobenzoates) nor a phenylurethane.

Oxidation. Potassium permanganate in acetone solution oxidized the lactols completely to the corresponding benzoic acids. Chromic acid in acetic acid did not react as rapidly; a 1,2-diketone was formed and separated from the unoxidized substance by crystallization. Some of the diketones were previously known. They were converted into quinoxalines by boiling with *o*-phenylenediamine in alcohol. The lactols (D, F) containing a piperonal

residue (R_2) on oxidation gave diketones that were so sensitive they could not be isolated. Even the *o*-phenylenediamine used in an attempt to get a quinoxaline was sufficiently alkaline to destroy them. In all instances, however, a deep violet-brown color was given with ferric chloride. A detailed description is given of the *p*-chlorlactol only.

In a small three-necked flask provided with a stirrer, thermometer, and dropping funnel, and surrounded by a cooling bath, were placed 20 gm. of the *p*-chlorlactol (VII A) and 150 cc. of glacial acetic acid, and a solution of 6 gm. of chromic acid in 15 cc. of acetic acid slowly admitted from the funnel, keeping the temperature below 30° C. After an hour the green solution was poured into water, extracted with ether and the extract well washed with water and dilute sodium carbonate solution. From the latter, on acidification, 3 gm. of a mixture of benzoic and *p*-chlorobenzoic acids were isolated, separated, and identified. The ethereal solution, on evaporation, left a residue of 10.6 gm.; it was taken up in methyl alcohol, filtered from a small amount of insoluble material* and recrystallized to a constant melting point of 103° C. A half-gram of the substance and an equal weight of *o*-phenylenediamine in 15 cc. of methyl alcohol was refluxed for five minutes, and precipitated by addition of water. On recrystallization, the quinoxaline melted at 132° C. These properties agree with those of benzyl *p*-chlorophenyl diketone (XV) as described by Jörlander (11). In a similar manner the other lactols were oxidized by chromic acid to yellow α-diketones. The properties are summarized in Table IV.

TABLE IV

PROPERTIES OF THE DIKETONES AND QUINOXALINES

Lactol used	Diketone					Quinoxaline			
	M.p. °C.	Form	Formula	Calcd. %	Found %	M.p. °C.	Formula	Calcd. %	Found %
A	103	plates	$C_{15}H_{11}O_2Cl$	C,69.6; H,4.2	C,69.5; H,4.2	132	Ref. 11		
B	161-5d.	needles	$C_{16}H_{11}O_4Cl$	Cl,11.7	Cl,11.6	161	$C_{22}H_{15}O_2N_2Cl$	Cl, 9.5	Cl, 9.8
C	122	plates	$C_{15}H_{11}O_2Br$			143	$C_{21}H_{15}N_2Br$	Br, 21.3	Br, 21.4
E	96	Ref. 11				138	Ref. 11		

Permanganate oxidation. Several oxidations were carried out, using 5 gm. of the lactol in 150 cc. of pure acetone, varying the amounts of permanganate and the temperature, but in every instance the result was a mixture of benzoic and *p*-chlorobenzoic acids (4.2 gm. in a typical case) which was separated into its components and identified by melting point and mixed melting points.

D. Properties of the chloride (XVI). The chloride was formed from the lactol and thionyl or acetyl chloride equally well. When 15 gm. of the *p*-chlorlactol was dissolved in an excess of the halide, warmed gently, and allowed to stand, 13.7 gm. of the solid chloride remained after the solvent was removed. After several recrystallizations from ether it formed glistening white needles,

**This solid, m.p. 202° C.; contained chlorine; only enough was obtained for one analysis. Found: C, 72.3; H, 4.2%—corresponding to $C_{22}H_{17}O_3Cl$. Since it was never isolated from other oxidations it was not further studied.*

m.p. 137° C. It is sparingly soluble in cold ether, insoluble in petroleum ether, and readily soluble in boiling ether, benzene, and acetone. Analysis: Calcd. for $C_{23}H_{16}O_2Cl_2$; Cl, 18.0%. Found: Cl, 17.8%. In a similar manner the *p*-bromlactol gave a chloride which crystallized in prisms, m.p. 132° C. Analysis: Calcd. for $C_{23}H_{16}O_2ClBr$: Cl, 8.1; Br, 18.2%. Found: Cl, 8.0; Br, 17.9%. It was impossible to get crystalline chlorides from the other lactols.

The acetate (*XVIII*). A mixture of 1.2 gm. of the *p*-chlor-chloride and 1 gm. of silver acetate in 15 cc. of absolute ether was refluxed an hour, filtered, and the ether allowed to evaporate. The residual solid was recrystallized from *n*-butyl alcohol; it formed shining, white, rectangular prisms, m.p. 157° C., insoluble in methyl and ethyl alcohols. Analysis: Calcd. for $C_{25}H_{19}O_4Cl$: Cl, 8.5%. Found: Cl, 8.6%. The same acetate was also formed by boiling for 3 min. 3 gm. of the lactol in 10 cc. of acetic anhydride containing a trace of sulphuric acid, pouring into ice water and extracting with ether; the yield was quantitative. A mixed melting point with the acetate above was not depressed.

Hydrolysis. A mixture of 0.5 gm. of the acetate, 50 cc. of methyl alcohol, and 9 cc. of concentrated ammonium hydroxide was left over night at 30° C. After neutralizing by addition of dilute acetic acid, crystals of the lactol separated. These showed no depression of the melting point when mixed with the lactol.

The methyl ether (*XVII*). A solution of 11 gm. of the chloride in 75 cc. of methyl alcohol was refluxed for two hours. On cooling, the methyl ether separated quantitatively, in dense white prisms, and was recrystallized from methyl alcohol, in which it is very soluble hot. Analysis: Calcd. for $C_{24}H_{19}O_3Cl$: CH_3O, 7.9%. Found: CH_3O, 7.6%.

Hydrolysis. On refluxing for 15 min. 1 gm. of the ether with 15 cc. of 10% methyl alcoholic potash and allowing to stand, the potassium salt of the acid (XX) separated, and was analyzed without attempting purification. Analysis: Calcd. for $C_{23}H_{16}O_3ClK$: K, 9.4%. Found: K, 8.8%. On adding acid to its aqueous solution, the lactol was precipitated and identified by a mixed melting point. The ether was not affected by ammonium hydroxide.

The methyl ether of the *p*-bromlactol was prepared in a similar manner from the corresponding chloride. It separated from methyl alcohol, in which it is only moderately soluble hot, as rectangular plates, m.p. 75° C. Analysis: Calcd. for $C_{24}H_{19}O_3Br$: CH_3O, 7.1%. Found: CH_3O, 7.2%. It was likewise hydrolyzed by alcoholic potash, the *p*-bromlactol precipitated by addition of acid, and its identity shown by a mixed melting point.

E. The methyl ester (*XIX*). The methyl ester could not be prepared by refluxing an alcoholic solution of the lactol and a trace of mineral acid (cf. Ref. 7) but was easily obtained through the silver salt.

Aqueous silver nitrate was added to 15 gm. of the lactol dissolved in an equivalent amount of sodium hydroxide solution, as long as a precipitate formed. The latter was filtered, and washed thoroughly with water, alcohol, and ether, and dried in a vacuum desiccator. Analysis: Calcd. for $C_{23}H_{16}O_3ClAg$:

Ag, 22.3; Cl, 7.4%. Found: Ag, 22.9; Cl, 7.6%. A suspension of 18.2 gm. of this silver salt in 75 cc. of absolute ether and 10 cc. of methyl iodide was refluxed 0.5 hr., filtered, and the solvent allowed to evaporate. There was left 7 gm. of ester, which after several recrystallizations from methyl alcohol formed short white needles, m.p. 87° C. It was only sparingly soluble in the cold alcohol but dissolved readily on being heated. Analysis: Calcd. for $C_{24}H_{19}O_3Cl$: Cl, 9.1%. Found: Cl, 9.1%.

Hydrolysis. Half a gram of the ester was refluxed with 10 cc. of 10% methyl alcoholic potash for 15 min., the solution neutralized with acetic acid and poured into water. The precipitated lactol was collected, recrystallized, and identified by a mixed melting point.

F. *The oxime (XXI).* The oxime of the *p*-chlorlactol (VII) was formed in the usual way in dilute alcoholic acetic acid, and purified by recrystallizing from dilute methyl alcohol. It formed short needles, m.p. 160° C., moderately soluble in cold alcohol and very soluble in hot alcohol and ether. Analysis: Calcd. for $C_{23}H_{18}O_3NCl$: N, 3.6%. Found: N, 3.5%.

References

1. ALLEN, C. F. H. J. Am. Chem. Soc. 47: 1733-41. 1925; 49: 1112-5. 1927.
2. ALLEN, C. F. H. and KIMBALL, R. H. Organic Syntheses. 10: 80-1. 1930.
3. BARAT, C. J. Ind. Chem. Soc. 7: 321-39. 1930; 8: 699-710. 1931.
4. BLAISE, E. E. and COURTOT, A. Bull. soc. chim. 35: 989-1004. 1906.
5. BORSCHE, W. Ber. 47: 1108-21, 2708-2722. 1914.
6. BREDT, J. Ann. 256: 314-340. 1890.
7. ERLENMEYER, E. JUN. and LUX, M. Ber. 31: 2224-38. 1898.
8. HANN, A. C. O. and LAPWORTH, A. J. Chem. Soc. 85: 1356-70. 1904.
9. HILL, H. B. Am. Chem. J. 3: 33-51. 1881.
10. HILL, H. B. and CORNELISON, R. W. Am. Chem. J. 16: 277-307. 1894.
11. JÖRLANDER, H. Ber. 50: 406-21. 1917.
12. KOHLER, E. P. and ALLEN, C. F. H. J. Am. Chem. Soc. 46: 1525-34. 1924.
13. KOHLER, E. P. and CHADWELL, H. M. Organic Syntheses. 2: 1-3. 1921.
14. KOHLER, E. P. and GOODWIN, R. C. J. Am. Chem. Soc. 49: 219-27. 1927.
15. KOHLER, E. P. and RICHTMYER, N. K. J. Am. Chem. Soc. 52: 3736-8. 1930.
16. KOHLER, E. P. and SHOHAN, J. B. J. Am. Chem. Soc. 48: 2425-34. 1926.
17. KON, G. A. R., STEVENSON, A. and THORPE, J. F. J. Chem. Soc. 121: 650-65. 1922.
18. LAPWORTH, A. and WECHSLER, E. J. Chem. Soc. 97: 38-48. 1910.
19. MEERWEIN, H. ET AL. J. prakt. Chem. 116: 229-75. 1927.
20. MEYER, H. Monatsh. 25: 491-9. 1904.
21. NORMINGTON, J. B. McGill University. Private communication.
22. QUDRAT-I-KHUDA. J. Chem. Soc. 128: 201-9. 1929; 129: 206-13. 1930; J. Ind. Chem. Soc. 8: 215-22. 1931.
23. ROTHSTEIN, E. and SHOPPEE, C. W. J. Chem. Soc. 126: 531-4. 1927.
24. ROTHSTEIN, E., STEVENSON, A. and THORPE, J. F. J. Chem. Soc. 127: 1072-80. 1925.
25. THIELE, J. ET AL. Ann. 319: 155-225. 1901.

THE EFFECT OF AGING ON THE ACTIVITY OF BAKER'S YEAST[1]

By R. K. Larmour[2] and S. F. Brockington[3]

Abstract

Yeast stored in cakes on ice showed little evidence of change in activity, as expressed in loaf volume, during the first 19 days; thereafter the loaf volume increased until the yeast was 30 days old; after this it decreased but never became as low as with the fresh yeast. The rate of carbon dioxide production was quite constant up to 26 days; thereafter it increased somewhat and maintained a higher though more irregular rate until the end of the series when the yeast was 56 days old.

Introduction

Flour is such a complex system of compounds that the task of assessing its value and of making accurate comparisons is very difficult and subject to large experimental error. Even in the case of a test such as the viscosity test, in which the added ingredients are simple chemical compounds and in which no manual technique is involved, slight changes in the conditions produce so profound an effect that the error of replication is large. The baking test involves manual technique which introduces still greater errors due to the personal factor of the operator. This factor has been extensively investigated by Geddes, Goulden, Hadley and Bergsteinsson (3) and by Merritt and Blish (6).

If all the ingredients used in bread making were simple chemical compounds such as water, salt and sugar, the errors observed could be attributed to manipulation, but another biological substance, yeast, must be included. Thus, there are two uncontrolled factors, the personal factor and the yeast factor, and as no one has yet been able to control the former it is very nearly impossible to segregate the variability that should properly be allocated to the latter. Whether or not the variability of the yeast contributes significantly to the total error of the baking test is a question still to be answered. The investigations of Werner and Siedhoff (7), Cook and Malloch (2), and Jorgensen (5) show that yeasts of different brands may vary considerably. Herman and Hart (4), on the other hand, tested two different brands of baker's yeast and found no appreciable difference in the baking results. Thus, all that can be said of different brands is that they may vary.

The product sold under one brand may also vary. Cook and Malloch (2), using eight samples of one brand observed the very large range, 289-416 cc., in the amount of carbon dioxide evolved in one hour. Another brand showed a range of 193-224 cc. for five samples. It is evident that careful factory

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control may result in a large reduction of variability but, on account of its nature, yeast would be expected to exhibit a certain amount of variation, even under the most favorable conditions of production.

Another source of variability is found in the shipment of the product from the plant to the distributor, and thence to the baker. This is important in western Canada because much of the commercial yeast used is produced on the Pacific coast, or in eastern Canada, and in either case it is several days old by the time the user receives it. Although great care is used in packing yeast for shipment it seemed possible that, in a country where wide extremes of temperature are common, some variability in activity might arise from this cause. An observation made in this laboratory confirmed the belief that the age of the yeast has a profound influence on its activity. In the course of a study of variability of loaf volume there was used a cake of yeast that had been in storage on ice for eight weeks. The baking results obtained with this and with freshly delivered yeast by one baker using the same flour are shown in graphs A and B, Fig. 1.

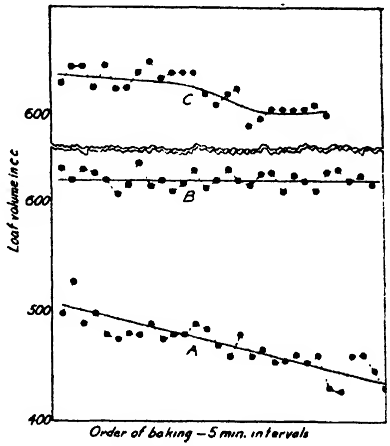

FIG. 1. *Three bakings made with one flour; A, with yeast eight weeks old; B, with yeast freshly delivered; C, with yeast three weeks old.*

The loaf volumes at the beginning of the series were lower than the values previously obtained with this flour and, as the baking progressed, there was a very marked decrease in volume until, at the end, the loaf volumes were approximately 75 cc. lower than at the beginning. This is not a usual occurrence although occasionally it has been suggested that there was a tendency for volume to fall off slightly toward the end of a run, as in graph C, Fig. 1. It was very important that definite information should be obtained on the behavior of yeast when kept in suspension at 30° C., because in our routine baking, yeast sufficient for 25 loaves is suspended at the beginning of the run and kept at 30° C. Furthermore, on the basis of the observation of Cook and Malloch (2) that yeast kept at 0° C. does not change appreciably in activity for periods up to 10 days, the authors had made a practice of having yeast delivered once a week and of storing it in contact with ice. It was necessary therefore to find out what changes might take place when yeast of varying age was kept suspended at 30° C.

Experimental

Series I

Twelve half-pound cakes of yeast were procured in one lot from the Winnipeg distributing plant and placed in cans on ice. Baking tests were made with a uniform sample of well aged, commercially milled, first patent flour, and all the bakings were performed by one operator. At each baking three doughs

were mixed at five-minute intervals with the freshly suspended yeast. The suspension was then stoppered and left on the panning bench until the end of the baking period, about 3½ hr., and shaken at intervals. At the end of this time three more doughs were mixed, using the old suspension. Averages of each set of three loaves are shown in Fig. 2. In this series of tests, 50 gm. of flour per loaf was used in place of 100 gm. During the first 11 days there was little change in the volume of loaves made with fresh suspension; with the exception of a slight decrease of about 8 cc. on the thirteenth day the volume was the same as on the first day, but thereafter a slight rise occurred and the loaf volume never fell below the 13-day value. The loaves made with the old suspension never equalled the others. The sharp drop in volume shown on the eleventh day seemed to be associated with high temperature of the yeast suspension. The container had been pushed back too close to the heaters and upon examination was found to be at a temperature of 42° C. The previous drop on the fourth and seventh days may have been due to the same cause. After the eleventh day the temperature of the suspension was checked frequently and kept very close to 30° C. After the twenty-first day, however, the loaves made with the old suspension dropped in volume and remained about 20 cc. lower than the check loaves. Disregarding the two drops previously mentioned, it appears that in the cases of both the fresh and old suspensions the activity tended to increase from the eleventh to the twentieth day, but the old suspension gave consistently lower values. After three weeks' storage on ice the yeast gave larger volumes than when it was fresh.

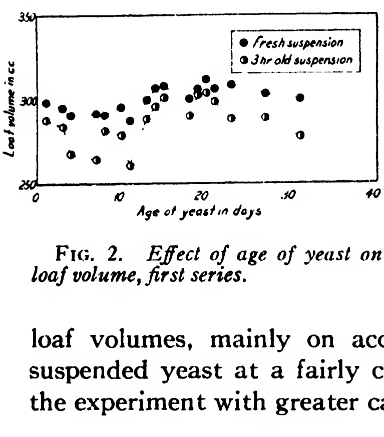

FIG. 2. *Effect of age of yeast on loaf volume, first series.*

Not satisfied with the results of this series of loaf volumes, mainly on account of the error due to failing to keep the suspended yeast at a fairly constant temperature, it was decided to repeat the experiment with greater care, and to make other tests of yeast activity.

TABLE I

EFFECT OF AGING OF YEAST ON LOAF VOLUME, STANDARD V FLOUR

Age of yeast, days	Loaf volume, cc.			Age of yeast, days	Loaf volume, cc.		
	Fresh suspension of yeast	Yeast suspension standing 3 hours at 30° C.	Mean		Fresh suspension of yeast	Yeast suspension standing 3 hours at 30° C.	Mean
7	650	635	642	29	687	673	680
12	653	632	642	33	683	710	697
14	653	640	646	41	670	657	664
19	652	675	664	49	670	658	664
26	663	660	662	56	685	688	686

Series II

For these experiments 12 half-pound cakes of yeast were procured in one lot. The number of replicate loaves was increased to five and the basic formula with 100 gm. of flour was used. Instead of storing the suspension on the bench it was kept in the proofing cabinet at 30 ± .5° C. during the interval between the first and second mixings. The average loaf volumes obtained are given in Table I and shown graphically in Fig. 3.

Usually the loaves made with the old suspension were smaller in loaf volume than those made with the fresh suspension, but there were two notable exceptions, namely, at 19 and at 33 days. On those dates the loaves made with the old suspension were unmistakably larger than those made with fresh suspension. In the last test, made with yeast 56 days old, there was no significant difference between the two sets of loaves. These data are inconclusive with respect to the effect of keeping yeast in suspension at 30° C. The average values were 667 and 663 cc. for the fresh and old suspensions respectively. It may be stated only tentatively that in most cases, the old suspension gave slightly lower loaf volume than the fresh suspension.

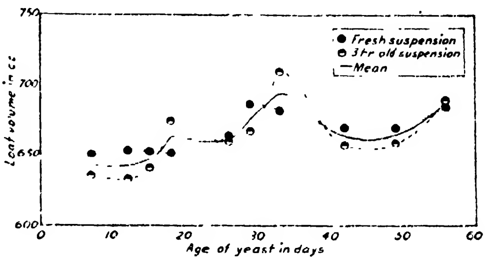

Fig. 3. *Effect of age of yeast on loaf volume, second series.*

In order to compare the trend of loaf volume with age of the yeast, the averages of all loaves baked in each test are shown as a curve in Fig. 3, each point representing the average volume of 10 loaves. It is unfortunate that there are not more data available for the period between 33 and 56 days, as the drop and final rise are rather peculiar and may be due partly to experimental error. Between 7 and 30 days the loaf volume increased with increasing age of the yeast. This improvement in volume was accompanied by improved bread characteristics. Interpreting the data broadly, it may be said that after the maximum at 30 to 33 days there was a gradual decrease in loaf volume, but it should be noted that in no case did the loaf volume drop below the volume observed at 7 days. The baking results with yeast varying in age from 7 to 15 days showed no significant variation and it is concluded, therefore, that for experimental baking yeast may be stored on ice for two weeks without undergoing any change detectable in the baking results.

Gas Production of Yeast of Various Ages

Concurrently with the baking tests, measurements were made of the gas produced in small doughs. These measurements were made by the method described by Bailey and Johnson (1), with certain minor modifications. The doughs were made up as follows: flour, 100 gm.; yeast, 3 gm; sugar, 2.5 gm.; salt, 1 gm.; water, 63 gm., and when taken from the mixer were quartered and placed in 150-cc. beakers. Two of these were placed in Mason jars containing

20% potassium hydroxide solution and two in jars containing 20% sodium chloride solution. As rapidly as possible the jars were immersed in a water bath at 30° C., and connected to the burettes. Readings were taken at 10-min.

TABLE II

EFFECT OF AGING YEAST ON GAS PRODUCTION IN DOUGH. RATE OF GAS PRODUCTION IN CC. PER 10 MIN. INTERVAL

Time interval	Age of yeast in days										
	7	12	14	19	26	29	33	41	49	56	Average
	Cc. of CO_2 produced in 10-min. intervals.										
10	5	4	5	4	4	4	4	3	6	2	4.1
20	7	8	12	8	7	8	8	6	8	4	7.6
30	13	12	12	10	10	10	11	10	12	6	10.6
40	14	14	15	15	12	14	12	12	13	9	13.0
50	15	16	15	16	14	16	14	11	13	9	13.9
60	16	15	16	16	13	16	12	13	14	11	14.2
70	15	15	16	15	14	18	16	13	13	10	14.5
80	18	15	16	17	15	16	16	14	16	11	15.4
90	15	17	16	16	15	16	15	16	16	13	15.5
100	15	15	15	17	15	16	15	15	16	14	15.3
110	15	14	15	16	14	17	14	13	14	13	14.5
120	14	14	16	15	14	16	16	14	15	13	14.7
130	15	15	13	13	13	16	14	13	15	14	14.1
140	15	13	14	16	14	16	15	14	14	13	14.4
150	13	13	13	14	13	16	14	14	13	13	13.6
160	13	13	14	13	13	14	14	15	13	12	13.4
170	11	13	13	13	11	15	14	15	15	12	13.2
180	12	12	12	12	12	13	15	14	14	14	13.0
190	11	12	13	13	12	14	14	12	14	12	12.7
200	13	12	11	12	11	12	13	13	13	12	12.2
210	12	11	12	12	12	13	12	11	13	14	12.2
220	11	12	11	11	11	13	12	13	13	12	11.9
230	13	13	12	13	11	12	12	12	13	11	12.2
240	10	11	12	11	11	12	12	12	13	14	11.8
250	12	10	10	11	11	12	14	12	12	11	11.5
260	10	11	11	11	12	13	12	12	13	12	11.7
270	11	11	10	11	11	11	12	11	11	12	11.1
280	10	10	10	10	11	12	12	12	13	12	11.2
290	11	10	11	11	10	12	11	12	11	11	11.0
300	10	9	10	11	10	11	12	10	13	12	10.8
310	10	10	10	10	10	10	11	11	11	13	10.6
320	9	10	10	10	10	11	12	11	10	12	10.5
330	9	9	9	10	10	10	12	10	11	9	9.9
340	10	9	10	10	9	10	11	10	10	12	10.1
350	9	10	10	10	9	10	10	10	9		9.7
360	10	9	9	10	10	10	9	10	11		9.8
370	7	8	8	9	10	9	10	9	10		8.9
380	10	8	9	10	9	8	10	9	9		9.0
390	10	9	9	10	9	9	9	10	10		9.4
400	7	9	8	9	9	8	9	9	8		8.4
410	8	7	8	8	8	7	7	9	8		7.8
420	8	8	8	8	9	6	7		7		7.6
430	8	6	8	8	8	7	8				7.6
440	8	8	7		8	6	7				7.3
450	7	7	7		7	6	7				6.8
460	7	7	7		7		6				6.8
470	7	7	6								6.7
480	6	6	6								6.0
490	6	6									6.0

intervals. This was carried out with freshly suspended yeast and with yeast that had been kept in suspension for 3½ hr. at 30° C. As there was no significant difference in gas production of these two, only the data obtained with doughs made from the freshly prepared suspensions have been reported in Table II.

In dealing with data concerning the production of carbon dioxide, it is customary to make graphs of total carbon dioxide produced, volume attained and carbon dioxide lost, plotted against time. In this experiment all these data were collected but, as we were interested in comparing samples of yeast rather than different flours, it was considered that it would be better to calculate rates of gas production. Accordingly, in Table II the data are presented as the volume of carbon dioxide produced in each 10-min. period, and thus by inspection the variation in rate of gas production may be determined. To facilitate comparison of the rates of gas production by yeasts of various ages, the average rate per 20 min. has been plotted against time in Fig. 4. These curves, with the exception of that at 56 days, are very similar in shape and indicate that the rate of gas production in doughs was little affected by aging for a period of 49 days. In all cases the rate increased rapidly at first, attaining a maximum of 15-17 cc. per 10 min. at the end of 70-90 min. Thereafter, the rate of gassing decreased somewhat rapidly at first and then more slowly until at the end of three hours it had fallen to 10-11.5 cc. per 10 min. The curve for 56 days differed considerably from the others in that the initial increase in rate was slower and did not reach such a high maximum, but at the end of the third hour this yeast was producing carbon dioxide at a rate higher than shown by the 7-day-old yeast.

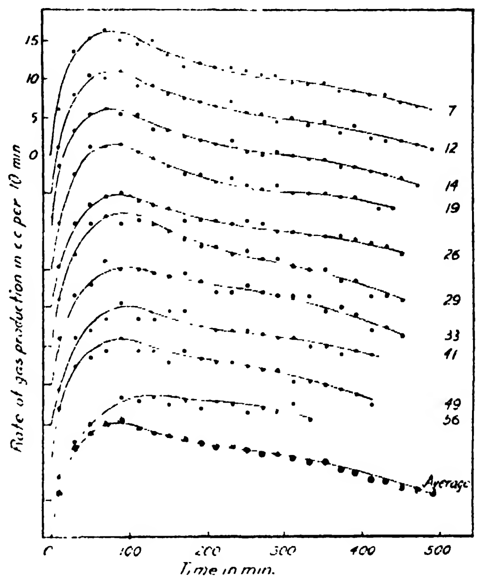

FIG. 4. *Rate of production of carbon dioxide by yeast of varying age.*

Probably the most crucial period in the dough is the proofing time, which in the authors' method is between 180 and 235 min. The rates of carbon dioxide production at the beginning and end of this period are shown in Table III. As the yeast became older there appeared to be an increase in rate of gas production during the period corresponding to the proof period. The average rate for the 60-min. interval, 180-240 min., was quite constant for the yeast samples ranging in age from 7 to 26 days; thereafter, in the 29-day-old sample there followed a rather abrupt increase. The last five samples, although showing considerable fluctuation in rate of carbon dioxide production during

this period on the whole produced gas at a greater rate than the first five of this series. The maximum rate for the series was obtained with the 49-day sample.

TABLE III

RATES OF CARBON DIOXIDE PRODUCTION AT THE TIMES CORRESPONDING TO THE BEGINNING AND END OF THE PROOFING PERIOD

Yeast sample, age in days	Rate of carbon dioxide production in cc. per 10 min. (from Table II)		Average	Yeast sample, age in days	Rate of carbon dioxide production in cc. per 10 min. (from Table II)		Average
	180 Min.	235 Min.			180 Min.	235 Min.	
7	11	10	11.7	29	14	12	12.6
12	12	11	11.6	33	14	12	12.7
14	13	12	11.6	41	12	12	12.1
19	13	11	11.9	49	14	13	13.0
26	12	11	11.3	56	12	14	12.3

The total amounts of carbon dioxide produced by these samples in periods of 3, 4 and 5 hr. are shown in Table IV. The values were fairly constant for all except the yeasts that were 29 and 56 days old, the former being higher and the latter lower than the others. The amount of carbon dioxide produced by the 49-day-old yeast was not significantly different from the amount produced by the yeast that was seven days old.

TABLE IV

TOTAL AMOUNT OF CARBON DIOXIDE PRODUCED BY THE VARIOUS YEAST SAMPLES IN PERIODS OF THREE, FOUR AND FIVE HOURS

Period of gas collection, hr.	Age of yeast, days									
	7	12	14	19	26	29	33	41	49	56
	Total amount of carbon dioxide produced, cc.									
3	241	238	248	246	223	257	239	226	240	191
4	311	309	319	318	291	333	314	299	309	266
5	375	370	381	383	356	404	387	368	382	336

There is little correspondence between the rate of gas production or total amount of carbon dioxide produced, and the loaf volume. The loaf volumes increased from 7 to 33 days and thereafter fell off. The rate of gas production was fairly constant up to 26 days and then increased and maintained a somewhat irregular higher level for the remainder of the samples. The total gas production was remarkably constant for all samples except those that were 26, 29 and 56 days old. The mean of the 26 and 29-day samples was in agreement with the others, but the 56-day sample was decidedly lower.

Conclusion

In conclusion it may be stated that, between 7 and 19 days, yeast stored on ice undergoes no change in rate or amount of carbon dioxide production, neither is there any significant difference in baking results. Thereafter, the loaf volume increases until the yeast reaches an age of 30 or 33 days, after which it decreases but the value never becomes as low as the value obtained with 7-day-old yeast.

The rate of carbon dioxide production in doughs is quite constant up to an age of 26 days but thereafter it increases somewhat and maintains a higher, though more irregular, level until an age of 56 days is attained by the yeast.

References

1. Bailey, C. H. and Johnson, A. H. Cereal Chem. 1: 293-304. 1924.
2. Cook, W. H. and Malloch, J. G. Cereal Chem. 7: 133-142. 1930.
3. Geddes, W. F., Goulden, C. H., Hadley, S. T. and Bergsteinsson, H. N. Can. J. Research, 4: 421-482. 1931.
4. Herman, R. S. and Hart, V. M. Cereal Chem. 4: 157-183. 1927.
5. Jorgensen, H. Cereal Chem. 8: 361-374. 1931.
6. Merritt, P. P. and Blish, M. J. Cereal Chem. 8: 267-292. 1931.
7. Werner, E. E. and Siedhoff, W. Cereal Chem. 6: 196-201. 1929.

STUDIES IN THE VARIABILITY OF TUBERCLE BACILLI

V. ACID AGGLUTINATION AND ELECTROPHORETIC POTENTIAL IN *MYCOB. LEPRAE*[1]

By G. B. Reed[2] and B. G. Gardiner[3]

Abstract

Previous work has indicated that various species of acid-fast bacteria including the tubercle bacilli may be separated into S and R types on the basis of colony structure and virulence; some results suggest that the two types differ in respect to the surface potential charge on the individual organisms. In this paper it is shown that S and R types of *Mycob. leprae* suspended in distilled water show a difference in electrophoretic potential of approximately five times the probable error of the determinations. Suspensions of S organisms are shown to have an isoelectric point of pH 1.2 compared with a pH of 2.2 in the case of a suspension of R organisms. Although acid agglutination of the S and R suspensions was found to occur at widely different pH levels, the agglutination occurred at approximately the same electrophoretic potential for both types, namely, at about 18.2 millivolts.

Introduction

In an early study of bacterial variation by DeKruif (1) two types of the bacillus of rabbit septicemia were differentiated by acid agglutination. Northrop (13) and Falk (3, 4, 5) recently reviewed the rather extensive literature, which has largely developed since that paper, dealing with variation from the point of view of acid agglutination, and particularly with the correlation between electrophoretic potential and virulence of strains within a species. It has generally been observed that cultures of a particular species showing the highest electrophoretic potential show also the greatest virulence. In some instances these two characteristics constitute the only observable differences, as in the case of the diphtheria bacillus, although this point has been questioned particularly by Jones (7). In other cases such as the *Pneumococcus* there appears to be a correlation between potential, serological types, and virulence for white mice. Where clear-cut S and R forms have been differentiated it has been observed in several instances that the S types, based on colony structure, show the higher virulence and the higher electrophoretic potential whereas the R forms are avirulent or of low virulence, and exhibit a lower potential.

Among the acid-fast species it was shown recently by Kahn and Schwarzkopf (8, 9), that S forms of tubercle bacilli show a higher electrophoretic potential than the R types. In two earlier papers in this series, Reed and Rice (14, 15), it was shown that the S and R forms from a considerable group of cultures of tubercle bacilli differ conspicuously in acid agglutination. These two results, apparently measurements of the same set of factors, suggested the desirability of a more detailed analysis in the hope of uncovering charac-

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teristics of variant types of acid-fast bacteria which would lend themselves to quantitative analysis. The results discussed in this paper are concerned entirely with S and R forms of *Mycob. leprae.*

It has just been shown (14) that *Mycob. leprae* as carried in stock cultures in various bacterial collections consists of S forms, R forms, or mixtures of the two, together with a variety of intermediate types. In fluid cultures the S forms were shown to be highly unstable and to dissociate readily to R. The R forms on the other hand proved relatively stable, yet R to S dissociation was demonstrated. In addition to the colony structure it was shown that the two types differed in habit of growth in fluid media, in oxidation-reduction potential changes during growth, and more particularly in complement fixation and agglutination reactions with specific S and R antisera. The S type was shown to contain a specific S antigenic substance, a species antigen, and an acid-fast group antigen, while the R form was shown to possess the two latter but to lack the specific S antigen.

Acid Agglutination

Well-established strains of S and R *Mycob. leprae* grown on either solid media such as glycerol-egg, or on fluid media such as Proskauer and Beck's fluid were removed with a minimum of media and ground in a mortar with distilled water to form an even suspension of the bacteria. This was readily accomplished by first grinding the semidry mass from the culture and then adding the water drop by drop until an even paste was produced. The S form always formed a stable suspension, while the R form required more grinding

TABLE I

ACID AGGLUTINATION OF SEVERAL S AND R CULTURES OF *Mycob. leprae* NO. 513. THE S AND R BRACKETED IN THE FIRST COLUMN REPRESENT CULTURES GROWN AND EXAMINED UNDER EXACTLY PARALLEL CONDITIONS. THE + SIGNS INDICATE THE DEGREE OF AGGLUTINATION

Organisms	pH of media												
	2.6	2.8	3.0	3.2	3.4	3 6	3.8	4.0	4.2	4.4	5.0	6.0	7.0
S 513	+ +	+	–	–	–	–	–	–	–	–	–	–	–
R 513	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	–	–	–	–	–
S 513	+ +	+ +	–	–	–	–	–	–	–	–	–	–	–
R 513	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	–	–	–	–	–
S 513	+ +	+	–	–	–	–	–	–	–	–	–	–	–
R 513	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	–	–	–	–	–
S 513	+ +	+ +	–	–	–	–	–	–	–	–	–	–	–
R 513	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	–	–	–	–	–
S 513	+ +	+	–	–	–	–	–	–	–	–	–	–	–
R 513	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	–	–	–	–
S 513	+ +	–	–	–	–	–	–	–	–	–	–	–	–
R 513	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+	–	–	–	–	–

and slower additions of water, but where these precautions were taken R suspensions could be made which remained stable for several hours. The suspensions were then washed three times by centrifuging and resuspending with grinding. Equal volumes of the suspensions and Clark's phosphate-phthalate buffer solutions ranging from pH 2.6 to 7.0 were mixed in small tubes. These were incubated for one hour in a water bath at 37° C. and the agglutination results read at once, and after 24 hr. at room temperature. The two readings were generally the same, the only difference being in the intensity of the agglutination.

Table I indicates the results obtained with several S and R cultures of *Mycob. leprae* No. 513. These were well-established types which had produced only S and R colonies, respectively, for several culture generations on solid media. The titration results are from cultures of ages varying from 10 to 30 days grown on gentian-violet glycerol-egg or in Proskauer and Beck's synthetic fluid. The bracketed pairs in the first column of the table represent S and R organisms cultured and examined under exactly parallel conditions. It will be observed that the pH at which agglutination occurred was uniform for each type.

Agglutination results with several well-established S and R types from other cultures of *Mycob. leprae* are shown in Table II.

TABLE II

ACID AGGLUTINATIONS OF S AND R TYPES OF *Mycob. leprae*. THE + SIGNS REPRESENT THE EXTENT OF THE AGGLUTINATION

Organisms	pH of buffer solutions									
	2.6	2.8	3.0	3.2	3.6	4.0	4.4	5.0	6.0	7.0
S type										
S 513	+ +	+ +	±	−	−	−	−	−	−	−
S 65	+ +	+ +	−	−	−	−	−	−	−	−
S 509	+ +	−	−	−	−	−	−	−	−	−
S 516	+ +	+	−	−	−	−	−	−	−	−
S 517	+ +	−	−	−	−	−	−	−	−	−
R type										
R 513	+ +	+ +	+ +	+ +	+ +	+ +	−	−	−	−
R 65	+ +	+ +	+ +	+ +	+ +	+ +	−	−	−	−
R 512	+ +	+ +	+ +	+ +	+ +	+	−	−	−	−
R 519	+ +	+ +	+ +	+ +	+ +	+ +	−	−	−	−
R 521	+ +	+ +	+ +	+ +	+ +	+ +	−	−	−	−

The values stated in Table II represent the average of several determinations, as do those in Table I. It is apparent that the S types from the several cultures are similar in this respect, and quite different from the R types which are similar to each other.

Measurements of Electrophoretic Velocity

Electrophoretic velocity determinations were made with the Northrop (13) type of apparatus. In the earlier experiments suspensions of organisms in distilled water were used. These were prepared as described in the preceding section. As it has generally been observed in such measurements that washing the organisms was essential, this procedure was followed in all the earlier experiments. It was found, however, that when the organisms were grown on solid media and carefully removed, washing did not influence the results. All readings were taken at the lower stationary level, as recommended by Falk (4), at a distance of $\frac{1}{3}D+\sqrt{3}$ from the lower inner surface of the cell. The cell was calibrated as described by Mudd (11).

Triplicate cultures of S and R forms of *Mycob. leprae* No. 513 were grown for six to seven days on glycerol-egg media, suspended in distilled water, washed, and the electrophoretic velocity determined. At least 10 organisms were timed in each preparation examined and from these readings the probable error was calculated. The results shown in Table III indicate an average velocity in microns per second per volt per centimetre of 4.36 for the S type and of 3.11 for the R type. The difference, 1.25, is approximately five times the probable error of the individual determinations.

TABLE III

ELECTROPHORETIC POTENTIALS OF SUSPENSIONS OF TYPICAL S AND R CULTURES OF *Mycob. leprae* NO. 513, SIX TO SEVEN DAYS OLD. THOSE BRACKETED WERE CULTURED AND EXAMINED UNDER PARALLEL CONDITIONS

Type of organism	Impressed voltage	Number of readings	Average time in seconds	Speed in μ/sec./volt/cm.
{S	126	10	7.46 ± .24	4.15 ± .13
{R	126	10	9.60 ± .42	3.22 ± .14
{S	126	10	7.05 ± .41	4.39 ± .26
{R	126	10	9.43 ± .64	3.28 ± .22
{S	126	10	6.81 ± .56	4.54 ± .37
{R	126	10	10.87 ± .75	2.84 ± .20
				Average S = 4.36 ± .25, Average R = 3.11 ± .19, Difference = 1.25.

A second group of cultures of both S and R types of varying ages were examined by the same procedure. The results tabulated in Table IV indicate that the electrophoretic potential increases gradually up to about three weeks, and later shows a decrease, especially in very old cultures. The average values, it will be observed, are much higher than those indicated in Table III for young cultures, however, approximately the same average difference between the S and R types is apparent.

Use of the Falk Cell

In an endeavor to simplify the procedure, the depression slide cell as used by Falk (4) was tried out. It proved to be less satisfactory than the Northrop-Kunitz type of apparatus especially on account of the polarization which caused water currents resulting in rapid changes in the readings. However, a number of sets of reasonably satisfactory readings are summarized in Table V. It will be observed that they are from 1 to 2 μ/sec./volt/cm. higher than those secured under comparable conditions with the Northrop-Kunitz apparatus, but the relative values are very similar and the conclusion the same; namely, that the S variant has a higher electrophoretic velocity and therefore a higher negative electrical charge than the R type.

TABLE IV

MEASUREMENT OF ELECTROPHORETIC VELOCITY OF S AND R TYPES OF *Mycob. leprae* FROM CULTURES OF VARIOUS AGES, WASHED AND SUSPENDED IN DISTILLED WATER

Age in days	S type		R type	
	Reading in seconds	Velocity in μ/sec./volt/cm.	Reading in seconds	Velocity in μ/sec./volt/cm.
6	7.11 ± .4	4.36 ± .25	9.97 ± .60	3.11 ± .19
8			8.75 ± .30	3.27 ± .11
11	5.6 ± .53	5.1 ± .48	8.00 ± .24	3.625 ± .11
14	4.9 ± .73	5.92 ± .88	7.75 ± .28	3.74 ± .13
15	5.37 ± .38	5.4 ± .38	8.3 ± .36	3 01 ± .13
24	4.4 ± .54	7.0 ± .86	5.1 ± .357	6.1 ± .42
28	5.6 ± .425	5.18 ± .375		
Average		5.52 ± .537		4.11 ± .18

TABLE V

A COMPARISON OF THE ELECTROPHORETIC VELOCITIES OF SUSPENSIONS OF FOUR TO SIX DAY CULTURES OF R AND S TYPES OF *Mycob. leprae* IN DISTILLED WATER, USING THE FALK CELL

Type of organism	Volts per cm.	Time for 420 μ in sec.	Velocity μ/sec.	Number of readings	Velocity in μ/sec./volt/cm.
S type					
S	24.3	3.2	131.2	10	5.4
S	24.3	3.2	131.2	10	5.4
S	24.3	3.2	131.2	10	5.4
S	24.3	3.3	128.1	10	5.3
Average S					5.38
R type					
R	24.3	3.9	107.7	10	4.4
R	24.3	4.1	102.4	10	4.2
R	24.3	4.0	105	10	4.3
R	24.3	4.3	97.6	10	4.0
Average R					4.22

TABLE VI

THE ELECTROPHORETIC VELOCITIES OF AN EIGHT-DAY R CULTURE OF *Mycob. leprae* WASHED AND SUSPENDED IN A SERIES OF BUFFER SOLUTIONS

	pH of buffer solutions									
	5.9	4.9	3.9	3.0	2.6	2.2	1.9	1.6	1.4	1.2
	Time in seconds									
	15	14.2	26.2	32			102.4	68.8	52	40.8
	14.	14	27	30.2			100.4	70.2	50.8	46.2
	13.2	13.8	23.4	31.8			104.2	66.0	48.2	44
	13.6	15.6	29.8	32	Almost no motion	No motion	96.0	78	58	38
	13	15	29.6	32			102.0	72.6	54	40.6
	13.6	16.2	26.4	29					62.4	50
	16.4	15	30.8	31.8					56.2	48.2
	15.8	15	25.8	30.2					61.8	42.4
	14.6	16.2	30.4	32					52.6	40
	13.2	16.4	32.6	29					52.2	48.2
Mean	14.24 ± .74	15.14 ± .60	28.2 ± 1.55	31.0 ± .81			101.0 ± 1.87	71.1 ± 2.72	63.3 ± 2.76	43.9 ± 2.6
μ/s/v/cm.	−2.01 ± .10	−1.9 ± .07	−1.01 ± .06	−0.29 ± .02			+.28 ± .005	+.4 ± .02	+.4 ± .03	+.65 ± .03

Determination of Isoelectric Points

Although the results indicated in the previous sections showed a definite difference between R and S types, it seemed from the work, particularly of McCutcheon, Mudd, Strumia and Lucké (10), that more striking results might be secured by a determination of isoelectric points. For this purpose a series of Clark's buffer solutions, ranging from pH 1.2 to pH 13 were prepared. Freshly washed bacterial suspension (2 cc.) was added to 20 cc. of the desired

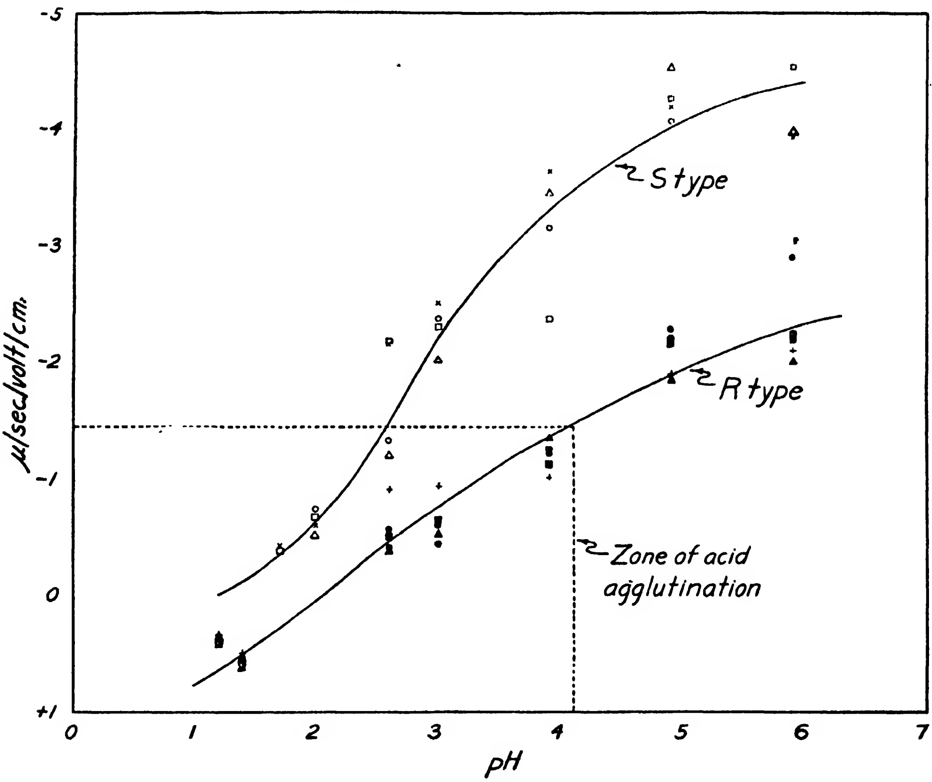

FIG. 1. *Curves of the electrophoretic potential of S (upper curve) and R (lower curve) Mycob. leprae suspended in buffer solutions of pH 1 to pH 7. The data are from Table VII :- S type;* × = 10 *day,* ○ = 14 *day,* □ = 15 *day,* △ = 28 *day cultures; R type;* + = 8 *day,* ● = 11 *day,* ▲ = 14 *day,* ■ = 20 *day,* ◑ = 41 *day cultures. The box enclosed by dash lines indicates the region of acid agglutination, data from Tables I and II.*

buffer mixture and electrophoretic readings were taken at once. More consistent results were obtained when the apparatus was flushed with a portion of the suspension in the buffer mixture just before readings were taken. It was soon found that there was no basic isoelectric point; attention was therefore focused on the acid end of the pH range. For this reason buffer solutions up to pH 7 only were used in most of the experiments.

Detailed results from the examination of an eight-day R type culture together with the calculated probable errors are shown in Table VI. It will

TABLE VII

Rate of migration of S and R *Mycob. leprae* suspended in buffer solutions at various pH values, arranged to show the isoelectric point. The numbers represent the speed in μ/sec./volt/cm.

Age of culture in days	pH 5.9	pH 4.9	pH 3.9	pH 3	pH 2.6	pH 2.4	pH 2.0	pH 1.7	pH 1.5	pH 1.4	pH 1.2
S type											
10	3.92 ± .16	4.20 ± .24	3.64 ± .18	2.50 ± .12	2.15 ± .09		.60 ± .030	.42 ± .026	Very slow	Slower	No motion
14	3.97 ± .15	4.08 ± .18	3.15 ± .11	2.38 ± .09	1.32 ± .11		.73 ± .037		Very slow	Slower	No motion
15	4.53 ± .29	4.26 ± .18	2.38 ± .19	2.30 ± .11	2.16 ± .07		.67 ± .010	.40 ± .030	Very slow	Slower	No motion
28	3.97 ± .18	4.53 ± .14	3.45 ± .13	2.01 ± .06	1.20 ± .03		.51 ± .018		Very slow	Slower	No motion
Average	4.10 ± .195	4.27 ± .185	3.15 ± .15	2.30 ± .095	1.71 ± .075		.63 ± .024	.41 ± .028	Very slow	Slower	No motion
R type											
8	2.10 ± .10	1.90 ± .05	1.01 ± .055	.92 ± .021	.90 ± .037	Very slow	No motion	Very slow		+ .5 ± .025	+ .65 ± .038
11	2.90 ± .21	2.28 ± .21	1.21 ± .054	.60 ± .023	.57 ± .035	Very slow	No motion	Very slow		+ .47 ± .015	+ .64 ± .027
14	2.00 ± .07	1.86 ± .06	1.35 ± .104	.52 ± .036	.38 ± .015	Very slow	No motion	Very slow		+ .37 ± .011	+ .58 ± .025
20	2.20 ± .17	2.16 ± .08	1.12 ± .080	.64 ± .03	.50 ± .023	Very slow	No motion	Very slow		+ .42 ± .014	+ .61 ± .026
41	2.34 ± .14	2.2 ± .02	1.24 ± .090	.44 ± .01	.40 ± .017	Very slow	No motion	Very slow		+ .40 ± .009	+ .58 ± .030
Average	2.29 ± .14	2.08 ± .08	1.19 ± .077	.624 ± .024	.55 ± .025	Very slow	No motion	Very slow		+ .43 ± .015	+ .61 ± .029

be observed that the electrophoretic velocity varied from 2.01 μ/sec./volt/cm. at a pH of 5.9 to zero at about pH 2.2. Below pH 2.2 the sign of the charge was reversed, and motion toward the cathode became more rapid with increasing acidity. Results from the examination of a series of both S and R cultures of *Mycob. leprae*, in the manner indicated in Table VI, are summarized in Table VII, the same results being shown graphically in Fig. 1. The R types show very consistently an isoelectric region about pH 2.2; in more acid solutions the sign of the charge is reversed, becoming positive. The S organisms on the other hand exhibit an isoelectric point in the region of pH 1.2. These characteristics have shown a remarkable constancy in the many cultures examined.

In the case of mixtures of R and S organisms suspended in buffers of pH 1.5 to pH 1.7, organisms may be seen to move in both directions. At these acidities the S organisms retain a negative charge and move, though slowly, toward the anode, while the R organisms assume a positive charge, and move toward the cathode.

Comparison of Electrophoresis and Acid Agglutination

Ellis (2) found that the stability of an oil-water emulsion was very closely connected with the potential difference between the oil drops and the surrounding medium. Northrop (12), using *B. typhosus* found a similar condition, *i.e.*, that agglutination occurred whenever the potential difference fell below 15 mv. provided that the cohesive force was not affected.

It has been shown, Table I, that the R type of *Mycob. leprae* agglutinates with great constancy at pH 4.0 to pH 4.1, while the S type agglutinates at pH 2.6 to pH 2.8. In Fig. I, as already noted, the results of a series of potential determinations of S and R types have been plotted with the potential in microns per second per volt per centimetre as ordinates and the pH of the menstruum as abscissa. The pH at which acid agglutination occurs has also been indicated on both the S and the R potential curves. It will be observed that though the R agglutinate at pH 4 and the S at pH 2.6 to pH 2.8 these points are at approximately the same potential, 1.4 μ/sec./volt/cm. Or applying the Lamb-Helmholtz equation $P.D. = \frac{4\pi uv}{Kx}$ which, as adopted by Northrop becomes $P.D. = 13 \times \mu$/sec./volt/cm., these results then show a critical *P.D.* of 18.2 millivolts, which is in substantial agreement with Northrop's findings that typhoid bacilli agglutinate when the *P.D.* falls to 15 mv. or lower.

In other words, while the two types agglutinate in the same potential zone, it requires a much more strongly acid condition to bring the S type to this potential than in the case of the R type.

Summary

1. S and R types of *Mycob. leprae* have been shown to be distinguishable by acid agglutination. The S type agglutinates in buffer solutions of pH 2.6 to pH 2.8, the R type in solutions of pH 4.0.

2. Electrophoretic potential determinations have indicated a similar type difference. With the organisms suspended in distilled water the potential difference between the two types amounted to some five times the probable error of the determinations.

3. Isoelectric point determinations have provided more precise and consistent differences. The isoelectric point of the S types was found to be at pH 1.2 and that of the R at pH 2.2.

4. Although the acid agglutination of S and R types was found to occur at widely different pH levels it was also observed to occur at approximately the same electrophoretic potential for both types, namely, at about 18.2 millivolts.

References

1. DeKruif, P. H. J. Gen. Physiol. 4: 387-393. 1922.
2. Ellis, R. Z. physik. Chem. 78: 321-352. 1912.
3. Falk, I. S., Gussin, H. A. and Jacobson, M. A. J. Infectious Diseases, 37: 481-494. 1925.
4. Falk, I. S., Jacobson, M. A. and Gussin, H. A. J. Infectious Diseases, 37: 499-506. 1925.
5. Falk, I. S., Jensen, L. B. and Mills, J. N. J. Bact. 15: 421-450. 1928.
6. Jensen, L. B. and Falk, I. S. J. Bact. 15: 413-419. 1928.
7. Jones, L. Proc. Soc. Exptl. Biol. Med. 28: 883-884. 1931.
8. Kahn, M. C. and Schwarzkopf, H. Proc. Soc. Exptl. Biol. Med. 27: 381-383. 1930.
9. Kahn, M. C. and Schwarzkopf, H. Am. Rev. Tuberc. 23: 45-55. 1931.
10. McCutcheon, M., Mudd, S., Strumia, M. and Lucké, B. J. Gen. Physiol. 13: 669-681. 1930.
11. Mudd, S. and Mudd, E. B. H. J. Exptl. Med. 46: 173-195. 1927.
12. Northrop, J. H. J. Gen. Physiol. 4: 629-633. 1922.
13. Northrop, J. H. See Jordan, E. O. and Falk, I. S. The newer knowledge of bacteriology and immunology. Univ. of Chicago Press. 1928.
14. Reed, G. B. J. Bact. In press.
15. Reed, G. B. and Rice, C. E. Can. J. Research, 4: 389-398. 1931.
16. Reed, G. B. and Rice, C. E. Can. J. Research, 5: 111-121. 1931.

A MATHEMATICAL THEORY OF THE GROWTH OF POPULATIONS OF THE FLOUR BEETLE, *TRIBOLIUM CONFUSUM*, DUV.[1]

By John Stanley[2]

Abstract

Biological data relative to the growth of populations of the Confused Flour Beetle, *Tribolium confusum*, Duv. have been examined mathematically, the individual insects being treated as moving or stationary particles amenable to the formulations of the kinetic theory of gases.

Under certain simplified conditions, *i.e.*, prior to the time of the first hatching of eggs, it was found possible to integrate the differential equations, obtaining curves showing substantial agreement with the biological data.

Beyond this point, a function $\theta(t)$ enters, the form of which has not as yet been determined, though further work on this point will be carried out. Therefore, at present, only a cursory discussion of the use of the function, etc., is given.

Such information as can be gained regarding the population growth in the later stages, without knowledge of the actual form of $\theta(t)$, is also given.

Introduction

The object of this investigation was to work out, as far as possible, a mathematical theory to explain the growth of populations of the Confused Flour Beetle, *Tribolium confusum*, Duv., living in whole wheat flour, the cultures to be made up and handled as hereafter described. This theory is then applied as far as possible to actual experimental data, to show how the various environmental and biological factors operate to force the growths of the populations investigated along the paths which they are found to follow.

Owing to the fact that all the biotic constants necessary have not yet been evaluated, exact numerical solutions cannot be given. Furthermore, owing to the complexity of the differential equations, many of them cannot be integrated as yet. Hence, the application of the theory must for the present be confined to the use of such information as can be obtained from an explanation of the population trends.

In order to handle the great number of variables and parameters, a numerical subscript notation has been used, whereby it is possible to determine at once to which life-history stage, etc., a given symbol refers.

The Confused Flour Beetle, *Tribolium confusum*, Duv. occurs commonly as a pest of stored products, grain, flour, cereals, etc., in various parts of the world. In the United States of America, examinations of such infested materials from the more northern states show an infestation consisting largely of this species, while in the more southern states, the species, *T. ferrugineum*,

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This paper was constructed from a thesis submitted to the Graduate Faculty of the University of Minnesota in partial fulfilment of the requirements for the degree of Doctor of Philosophy, the work being done partly at the University of Minnesota, and partly at the Experiment Station of the Association of Hawaiian Pineapple Canners, Honolulu.

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Fab., is more likely to be found. The two species are almost identical in habits and form, and the theories set forth herein can be applied equally well to populations of *T. ferrugineum* Fab., with suitable changes in the values of the biotic constants.

General Statement of the Habits and Life History

The adults of *T. confusum*, Duv. are small brown beetles on an average 3.40 mm. long by 1.02 mm. wide, Table VII. The females, which form 50% of the adult population, lay small, white, ovoid eggs, at a rate which depends on the temperature, Table II. After a period of from 4 to 40 days, Table I,

TABLE I*

BIOTIC CONSTANTS OF *T. confusum* AT 75% RELATIVE HUMIDITY, AND AT THE TEMPERATURES INDICATED

Form	Expt.	Time in days	Mean time A and B	Standard deviation	Probable error	Coefficient of variability (mean)
32°C.						
Eggs	A†	4.42		0.136	0.092	
	B	4.42	4.42	0.137	0.093	3.093%
Larvae	A	17.38		0.773	0.521	
	B	17.30	17.34	0.744	0.501	4.372%
Pupae	A	5.32		0.716	0.482	
	B	5.44	5.38	0.694	0.467	13.105%
27°C.						
Eggs	A	6.05		0.130	0.088	
	B	6.03	6.04	0.105	0.071	1.950%
Larvae	A	22.33		0.634	0.427	
	B	22.51	22.42	0.511	0.345	2.557%
Pupae	A	8.72		0.871	0.588	
	B	8.57	8.69	0.738	0.497	9.305%
22°C.						
Eggs	A	14.10		0.261	0.176	
	B	14.08	14.09	0.237	0.160	1.760%
Larvae	A	60.49		4.851	3.273	
	B	61.73	61.11	4.875	3.289	7.959%
Pupae	A	16.94		0.885	0.597	
	B	18.78	17.86	2.078	1.402	8.645%
17°C.						
Eggs	A	38.80		0.980	0.661	2.000%
	B	38.83	38.82	0.897	0.534	2.418%

* *The author is indebted to Dr. R. N. Chapman for the data of Table I.*
† *These are not the A's and B's of Tables X to XIII.*

TABLE II*

EGG LAYING RATE OF *T. confusum* AT VARIOUS TEMPERATURES

Temp. °C.	Mean rate per ♀ day	Standard deviation	Probable error	Coefficient of variability
17	—	—	—	—
22	1.90	1.181	.797	62.234%
27	6.24	1.667	1.124	26.622%
32	10.73	2.887	1.948	26.912%

* *The writer is indebted to Dr. R. N. Chapman for the data of Table II.*

TABLE III

DIMENSIONS OF THE EGGS OF *T. confusum* *

Dimension	Min., mm.	Max., mm.	Mean, mm.	Stand. dev.
Data from Stanley (100 measured)				
Length	.52	.80	.64	.05
Width	.32	.50	.40	.02
Data from Brindley (3) (25 measured)				
Length	.62	.73	.64	.04
Width	.38	.47	.40	.02

* *With adherent flour.*

depending on the temperature, these eggs hatch to minute whitish larvae which wander in the flour and, during the course of their lives, moult their skins six times, increasing in size and vigor with each moult. At the last moult they change to the non-motile pupae. This series of changes takes from 17 to 61 days, again depending upon the temperature, as will be seen from Table I. The pupae remain motionless except for a slight wiggling movement for from 5 to 18 days, once more depending upon the temperature, Table I, and then, on the splitting of the skin, the new adult emerges. The new female adult does not lay eggs for a short time, the pre-oviposition period, which curiously enough is almost the same as the duration of the egg stage.

During life the adults and larvae of various ages wander about in an apparently aimless way in the flour, the adults, if female, laying eggs *en route*. Both adults and larvae are cannibalistic, living partly upon flour and partly upon such eggs, smaller larvae, and pupae as they can find and eat. There is no evidence, however, that they purposely search for this living food. They appear to be satisfied with that which is offered to them as a result of their movement through the flour. They require almost no care to rear, beyond seeing that the flour does not become filled with excreted waste.

TABLE IV

MISCELLANEOUS DATA FOR EGGS (STANLEY)

Weight of egg, 10,000 weighed (*en masse*), gm., 0.000,057,8 = W_2,(27°C).
Moisture in egg, mean of 3 samples of 10,000 each, 44.958%.
Available nutrient material in egg, gm. 0.000,025 = A_2
Percentage of eggs to hatch, 27°C. = 90, U_2 = .9

TABLE V

STADIA OF LARVAE OF *T. confusum* AT 27°C.

Stadium	First	Second	Third	Fourth	Fifth	Sixth
Length, days	2.43	3.63	3.03	3.27	3.39	6.67

NOTE:—*Values computed from Brindley's (3) data for 29.7°C.*

TABLE VI

WEIGHT OF ADULT BEETLES OF *T. confusum* FROM BRINDLEY (3), 80 OF EACH SEX WEIGHED

Sex	Min. wt., gm.	Max. wt., gm.	Mean wt., gm.	Stand. dev.
Male	.00140	.00155	.00148	.00006
Female	.00174	.00188	.00178	.00006

From the above general statement, it will be seen that *T. confusum* Duv. offers certain distinct advantages as a laboratory animal for population studies. It makes no webs or other structures in the flour, it is fairly resistant to handling, can be sifted from the flour to make population counts, and can live and develop over a wide range of temperature. For these reasons it has been used to some extent as an experimental animal, as the following survey of the literature will show.

TABLE VII

DIMENSIONS OF ADULT BEETLES OF *T. confusum* FROM BRINDLEY (3), 50 OF EACH SEX MEASURED

Dimension	Min., mm.	Max., mm.	Mean, mm.	Stand. dev.
Length	3.15	3.83	3.40	.14
Width	.85	1.11	1.02	.05

A Survey of the Literature

We shall confine ourselves in this survey to papers dealing with habits, life history, population growth, etc., as purely taxonomic papers are not of paramount importance with regard to the problem in hand.

The most important paper with regard to the insect is no doubt that of Chapman (7), in which he shows that the growth of a population of *T. confusum*, is dependent, at any one temperature, upon the size of the environment (a dish of flour), and upon the initial concentration of the beetles, but that the final concentration is independent of either the initial concentration or the size of the environment. Others have checked this work, (See Allee (1), Park (11)) and have obtained substantially the same results.

That there may be under certain conditions other limiting factors than eating is shown by the paper of Chapman (6), in which he speaks of a pungent gas given off by the beetle when irritated, and which causes the production of monstrosities if present in relatively small concentrations in the air around mature larvae. That this limiting factor is not considered in the following theory is due to the fact that monstrosities were seldom if ever observed, as the populations were always very carefully handled.

The life history has been worked out by Chapman (4) and by Brindley (3). The insect is also mentioned by Chapman (5).

Its nutritional requirements with regard to vitamins have been studied by Sweetman and Palmer (12).

Further studies have been made upon its life history by Holdaway (9), with regard to the production of intersexes, and finally there is the work of Gause (8)

in which he shows how changes in the various environmental factors may be correlated with the various equilibrium values assumed by the populations.

Four other papers worthy of mention, as they represent what is up to the present the best mathematical work on the subject of population growth, are those of Volterra (13, 14, 15), and Bailey (2).

The writer feels that some criticism may be levelled against Volterra's work on the grounds that so many assumptions have been made in order to simplify the mathematical treatment, that the entities considered can nowhere be found in the roster of living organisms.

Although the population counts on which Figs. 2 to 9, and the theory itself are based were made, not by the writer, but by Dr. R. N. Chapman, and his assistants, an explanation of the technique used would seem desirable.

Briefly then, whole-wheat flour was sifted through No. 8 silk bolting cloth to obtain an homogeneous fine flour containing sufficient vitamins for growth. This flour was then placed in the controlled temperature cabinets for a few days so that it might come into temperature and relative humidity equilibria with the air in the cabinets, and was then weighed out in lots having a weight of 32 gm. at 27° C., and 75% relative humidity (air). Previous to this, a number of adults were caged and the egg laying rate of each female carefully determined. Since violent shaking or sifting was found to alter the egg laying rate, these determinations were made by caging single females, and carefully rolling them out of the flour with a minimum of disturbance. The eight females for each duplicate lot were then selected to avoid the inclusion of any females having egg laying rates differing widely from the mean.

Eight males and eight of the selected females were then placed in each beaker with 32 gm. of flour and placed in the cabinets.

Counts were in general made every 10 days, the various life-history stages being separated by the use of bolting cloth sieves of various meshes. It was found that repeated counting at intervals of less than 10 days seriously altered the egg laying rate.

The flour was changed at each count, and on returning the beetles to the flour, great care was used to distribute them evenly through the mass.

Thus small controlled environments were set up in which the beetles lived and grew, and, provided care was taken to exclude certain parasitic mites and intestinal parasites, no trouble was experienced in obtaining parallel results with duplicate cultures. It was this close agreement between duplicate cultures that led to the following investigation subsequent to Dr. Chapman's pointing it out to the writer.

Part 1.

The Population Growth Under Conditions Such That Environmental and Biotic Resistance are Zero

It will be apparent that differential equations can be written, descriptive of the growth of the population, by the use of parameters descriptive of the insects and the environment.

In the following discussion the symbols pertaining to the various materials, flour and the different life-history stages, will be identified by the use of the following numerical subscripts, in accordance with the scheme outlined in the introduction.

Flour, 1; eggs, 2; first instar larvae, 3; second instar larvae, 4; third instar larvae, 5; fourth instar larvae, 6; fifth instar larvae, 7; sixth instar larvae, 8; pupae, 9; immature adults, *i.e.*, adults which have not passed the pre-oviposition period, 10; mature adults, 11.

Since however the point which we desire to make in Part I can be proved without a consideration of the various instars, etc., there is given below only a limited notation, sufficient for the matter in hand.

Let: N_0 = original number of adults.
N_2 = number of eggs at any time, T.
R = sex ratio, *i.e.* ratio of females to total.
ϵ = number of eggs laid per female per unit time.
T_0 = origin of time, *i.e.*, time at which population is set up.
$t_2(M)$ = time at which the laying of eggs of the M[th] generation commences.
$t_3(M)$ = time at which the hatching of eggs of the M[th] generation commences.
Γ_2 = number of days spent in the egg stage.
S = total time from egg to egg.

We shall now, under Part I, consider the idealized growth of a population, *i.e.*, on the supposition that there is no environmental resistance, no cannibalistic eating, and no variation in the values of the biotic or environmental parameters.

It will be seen, after careful consideration of the matter that, in any generation, there are two phases. For example, in the M^{th} generation there are the following:– (a) *Phase* M_1—from the commencement of laying by the M^{th} generation adults to the time of hatching of their eggs, *i.e.*, during the period of time, $t_2(M) < T < t_3(M)$. (b) *Phase* M_2—from the commencement of hatching of the M^{th} generation eggs to the commencement of laying of the $(M+1)^{th}$ generation eggs, that is during the period, $t_3(M) < T < t_2(M+1)$.

The equations descriptive of the growth of the egg population during the various phases are as follows.

Phase 1_1

This phase covers the time interval, $t_2(1) < T < t_3(1)$, *i.e.*, from the commencement of laying of first-generation eggs, to the commencement oftheir hatching.

During this period,

$$N_2 = R\epsilon N_0 T$$

Where N_0 is the original number of adults, not necessarily 16, as in the experiments performed in connection with this particular problem.

Phase 1_2

This phase covers the time interval $t_3(1) < T < t_2(2)$, *i.e.*, from the commencement of hatching of first-generation eggs to the commencement of laying of

second-generation eggs. During this phase, and during all subsequent time, the rate of increase of eggs is diminished by the hatching of first-generation eggs. The number of first-generation eggs which have hatched up to a time T is the number which were laid up to a time $(T-\Gamma_2)$, that is,

$$R\epsilon N_0(T-\Gamma_2).$$

Whence, during Phase 1_2

$$N_2 = R\epsilon N_0 T - R\epsilon N_0(T-\Gamma_2) = R\epsilon N_0\Gamma_2 = \text{constant}.$$

Phase 2_1

This phase covers the time interval, $t_2(2) < T < t_3(2)$, *i.e.*, from the commencement of laying of second-generation eggs to the commencement of their hatching. During this period, the rate of change of N_2 is increased by the production of second-generation eggs which are laid by adults formed from first-generation eggs laid during the period $T_0 < t < T-S$, where S is the time necessary for transformation of a newly laid egg to a fully mature adult. The group of N_0R first-generation eggs laid at $T = T_0 + \frac{1}{\epsilon}$ will mature and lay eggs in turn at $T = S + \frac{1}{\epsilon}$ and will lay $N_0R^2(T-S)\epsilon$ second-generation eggs up to a time T.

The next group, laid at $T = S + \frac{2}{\epsilon}$ will in turn produce second-generation eggs to the number of $N_0R^2[(T-S)\epsilon - 1]$ up to a time T. Thus the total number of second-generation eggs laid subsequent to a time, S, and up to a time T, will be, including the last group,

$$N = N_0R^2\left\{(T-S)\epsilon + [(T-S)\epsilon - 1] + [(T-S)\epsilon - 2] + \ldots [(T-S)\epsilon - (T-S)\epsilon]\right\}$$

$$= \sum_{\alpha_1=0}^{\alpha_1=(T-S)\epsilon} N_0R^2[(T-S)\epsilon - \alpha_1].$$

Whence, during Phase 2_1, the total number of eggs at any time T, during the period of the phase is,

$$N_2 = R\epsilon N_0\Gamma_2 + \sum_{\alpha_1=0}^{\alpha_1=(T-S)\epsilon} N_0R^2[(T-S)\epsilon - \alpha_1].$$

Phase 2_2

This phase covers the time interval, $t_3(2) < T < t_2(3)$, *i.e.*, from the commencement of hatching of second-generation eggs to the commencement of laying of third-generation eggs. During this phase and after, a negative term enters due to the hatching of the second-generation eggs. The number which hatch up to a time T is equal to the number laid up to a time $(T-\Gamma_2)$, that is,

$$\sum_{\alpha_1=0}^{\alpha_1=(T-S-\Gamma_2)\epsilon} N_0R^2[(T-S-\Gamma_2)\epsilon - \alpha_1].$$

Whence, at any time, T, during this phase,

$$N_2 = R\epsilon N_0\Gamma_2 + \sum_{\alpha_1=0}^{\alpha_1=(T-S)\epsilon} N_0R^2[(T-S)\epsilon - \alpha_1] - \sum_{\alpha_1=0}^{\alpha_1=(T-S-\Gamma_2)\epsilon} N_0R^2[(T-S-\Gamma_2)\epsilon - \alpha_1].$$

Phase 3_1

This phase covers the time interval $t_2(3) < T < t_3(3)$, *i.e.*, from the commencement of laying of third-generation eggs to the commencement of their hatching. In a manner analogous to that in which the second-generation eggs discussed under Phase 2_1 were formed, these third-generation eggs are formed from eggs laid during a time subsequent to $T = 2S$.

The second-generation eggs laid at a time $T = 2S + \frac{1}{\epsilon}$ are equal to N_0R^2, and, upon transformation to adults, will lay third-generation eggs to the number of $N_0R^3[(T-2S)\epsilon]$, up to a time T.

The second group of second-generation eggs laid in the interval $2S + \frac{1}{\epsilon} < T < 2S + \frac{2}{\epsilon}$ are, from the calculations of Phase 2_1, equal to $\sum_{i=1}^{i=2} R^2 i$ and lay third-generation eggs to the number of $\sum_{i=1}^{2} N_0R^3 i[(T-2S)\epsilon - 1]$ up to a time T.

Whence, by a continuation of the above reasoning, the total number of third-generation eggs laid up to a time T is,

$$N = \sum_{i=1}^{i=1} N_0R^3 i[(T-2S)\epsilon - 0] + \sum_{i=1}^{i=2} N_0R^3 i[(T-2S)\epsilon - 1] + \sum_{i=1}^{i=3} N_0R^3 i[(T-2S)\epsilon - 2] + \ldots\ldots\ldots + \ldots\ldots \sum_{i=1}^{i=(T-2S)\epsilon} N_0R^3 i\left[(T-2S)\epsilon - \left(T-2S-\frac{1}{\epsilon}\right)\epsilon\right]$$

whence, $$N = \sum_{\alpha_2=1}^{\alpha_2=(T-2S)\epsilon} \sum_{\alpha_1=0}^{\alpha_1=\alpha_2} N_0R^3[(T-2S)\epsilon - \alpha_1].$$

Whence, during the interval, $t_2(3) < T < t_3(3)$

$$N_2 = R\epsilon N_0\Gamma_2 + \sum_{\alpha_1=0}^{\alpha_1=(T-S)\epsilon} N_0R^2[(T-S)\epsilon - \alpha_1] - \sum_{\alpha_1=0}^{\alpha_1=(T-S-\Gamma_2)} N_0R^2[(T-S-\Gamma_2)\epsilon - \alpha_1] + \sum_{\alpha_2=1}^{\alpha_2=(T-2S)\epsilon} \sum_{\alpha_1=0}^{\alpha_1=\alpha_2} N_0R^3[(T-2S)\epsilon - \alpha_1]$$

Phase 3_2

This phase covers the time interval, $t_3(3) < T < t_2(4)$, *i.e.*, from the commencement of hatching of third-generation eggs to the commencement of laying of fourth-generation eggs. By an extension of the reasoning employed under Phase 2_2, the additional negative term is

$$-\sum_{\alpha_2=1}^{\alpha_2=(T-S-\Gamma_2)\epsilon} \sum_{\alpha_1=0}^{\alpha_1=\alpha_2} N_0R^3[(T-S-\Gamma_2)\epsilon - \alpha_1].$$

Phase 4_1

This phase covers the time interval, $t_2(4) < T < t_3(4)$ *i.e.*, from the commencement of hatching of fourth-generation eggs to the commencement of

laying of fifth generation eggs. By an extension of the reasoning used under Phase 3_1, the additional positive term is

$$\sum_{\alpha_3=1}^{\alpha_3=(T-3S)\epsilon} \sum_{\alpha_2=1}^{\alpha_2=\alpha_3} \sum_{\alpha_1=0}^{\alpha_1=\alpha_2} N_0R^4\left[(T-3S)\epsilon-\alpha_1\right].$$

Phase 4_2

This phase covers the time interval, $t_3(4)<T<t_2(5)$, *i.e.*, from the commencement of laying of fifth-generation eggs to the commencement of their hatching. Again a negative term enters, which can be shown to be

$$-\sum_{\alpha_3=1}^{\alpha_3=(T-S-\Gamma_2)\epsilon} \sum_{\alpha_2=1}^{\alpha_2=\alpha_3} \sum_{\alpha_1=0}^{\alpha_1=\alpha_2} N_0R^4\left[(T-3S-\Gamma_2)\epsilon-\alpha_1\right].$$

Whence, for phase M_1 where $t_2\,(M)<T<t_3(M)$

$$N_2=R\epsilon N_0\Gamma_2+\sum_{\alpha_1=0}^{\alpha_1=(T-S)\epsilon} N_0R^2\left[(T-S)\epsilon-\alpha_1\right]-\sum_{\alpha_1=0}^{(T-S-\Gamma_2)\epsilon} N_0R^2\left[(T-S-\Gamma_2)\epsilon-\alpha_1\right]$$

$$+\sum_{\alpha_2=1}^{\alpha_2=(T-2S)\epsilon} \sum_{\alpha_1=0}^{\alpha_1=\alpha_2} N_0R^3\left[(T-2S)\epsilon-\alpha_1\right]-\sum_{\alpha_2=1}^{\alpha_2=(T-2S-\Gamma_2)\epsilon} \sum_{\alpha_1=0}^{\alpha_1=\alpha_2} N_0R^3\left[(T-2S-\Gamma_2)\epsilon-\alpha_1\right]$$

$$+\ldots\ldots-\ldots\ldots+\ldots\ldots-\ldots\ldots+\ldots\ldots-\ldots\ldots+$$

$$+\sum_{\alpha_{(M-1)}=1}^{\alpha_{(M-1)}=[T-(M-1)S]\epsilon} \sum_{\alpha_{(M-2)}=1}^{\alpha_{(M-2)}=\alpha_{(M-1)}} \cdots\cdots\cdots \sum_{\alpha_{(M-i)}=1}^{\alpha_{(M-i)}=\alpha_{(M-i+1)}} \cdots\cdots\cdots$$

$$\cdots\cdots\cdots+\sum_{\alpha_2=1}^{\alpha_2=\alpha_3} \sum_{\alpha_1=0}^{\alpha_1=\alpha_2} N_0R^{(M-1)}\left[\left\{T-(M-1)S\right\}\epsilon-\alpha_1\right].$$

During Phase M_2, where $t_3(M)<T<t_2(M+1)$ the additional negative term is

$$-\sum_{\alpha_{(M-1)}=1}^{\alpha_{(M-1)}=[T-(M-1)S-\Gamma_2]\epsilon} \sum_{\alpha_{(M-2)}=1}^{\alpha_{(M-2)}=\alpha_{(M-1)}} \cdots\cdots\cdots \sum_{\alpha_{(M-i)}=1}^{\alpha_{(M-i)}=\alpha_{(M-i+1)}} \cdots\cdots\cdots$$

$$\cdots\cdots\sum_{\alpha_2=1}^{\alpha_2=\alpha_3} \sum_{\alpha_1=0}^{\alpha_1=\alpha_2} N_0R^{(M-1)}\left[\left\{T-(M-1)S-\Gamma_2\right\}\epsilon-\alpha_1\right].$$

By an extension of the above method we may write equations descriptive of the growths of populations of any life-history stage.

The above equations demonstrate two important facts:

(a) That a population of this type, having distinct generations does not increase according to the compound interest law. It could probably be shown that such a law of increase does hold when T becomes very great, or if S is very small, as for instance in the case of bacteria and protozoa.

(b) That the population existing at any time is a function of its whole past history. Hence damage to a population is, theoretically at least, irreparable within finite time. In actual populations, owing to the action of environmental resistance, damaged populations do tend to return to the form which they would have had if undamaged, but never actually reach it.

Part 2

Population Growth Under Conditions Such That Environmental and Biotic Resistance are Greater Than Zero

The population growth will now be considered under the assumptions that temperature, relative humidity and the weight and chemical constitution of the flour are constant, while the various subtractive forces, resulting from natural mortality and from the eating of one form by another, are allowed full play.

These assumptions bring a very complicated system of forces into existence, hence the following general statement, supplementing that already given, may be of interest.

The original 16 adults, eight males and eight females, are placed in the flour and at once commence to lay eggs. At the same time, as a result of their movement through the flour, they again encounter some of their own eggs, and may eat them, certain conditions being satisfied. As a result of this eating and laying, the eggs tend to increase to the point where they are so numerous as to be found and eaten as rapidly as laid. Flour is of course eaten at the same time, a certain mathematical relationship existing between the amounts of egg material and flour consumed per adult per unit time.

Subsequent to hatching, the new larvae themselves prey upon the eggs, consume flour, and are in turn preyed upon by each other and by the adults. Young larvae also fall victims to older larvae.

At the time of the first pupation, the larval population is temporarily decreased by transformation to the pupal stage. At the same time, the egg population rises owing to the fact that egg-eating larvae are being transformed to non-egg-eating pupae. Thus at this point, except under certain special conditions to be discussed later (see page 666) the egg and pupal populations are increasing, and the larval population is decreasing.

Upon the commencement of emergence to the adult form, the passive pupae are changed over to voracious egg-eating adults, as yet too immature to lay eggs. Consequently, egg, larval, and pupal populations tend to decrease, while the adult population increases.

After a certain time, the pre-oviposition period, the oldest of the new adults *i.e* , the oldest female, begins to lay, and from this point on, the egg population increases to come into periodic fluctuating equilibrium with all the other forms. This increase in number of eggs is reflected in a later increase in larvae, and a still later and smaller increase in pupae. Occasionally there may be a later and small increase in adults. The eating of one form by another exerts a damping influence on the amplitude of these small increases. Figs. 4 to 9 will show the general truth of the above statements.

The problem of the numbers of contacts in unit time, occurring between insects moving in the flour

In order to discuss the manifold forces acting within the population, it is necessary to have some means of computing the rate at which contacts occur between the various individuals moving in the flour, since such contacts may result in the eating of some individuals by others.

If the individual insects be considered merely as moving particles, and owing to the almost infinitesimal degree of intelligence possessed, it is legitimate to make such an assumption, the problem may be handled by means of formulas from the kinetic theory of gases.

This theory (10) shows that three types of contacts may occur between particles moving in a given space: (a) contacts between a moving particle and a stationary particle, (b) contacts between two particles moving with the same speed, and (c) contacts between two moving particles having different speeds.

Thus, under case (a), where V is the volume of the space through which the particles are distributed, ν is the number of stationary particles, σ_1 their mean diameter, σ_2 the diameter of the moving particle, and μ its mean speed, the number of contacts in unit time between the moving particle and the ν stationary particles is,

$$N_a = \frac{\mu \pi \nu \left(\frac{\sigma_1 + \sigma_2}{2} \right)^2}{V - \frac{4}{3} \pi \nu \left(\frac{\sigma_1 + \sigma_2}{2} \right)^3} . \tag{1}$$

Under case (b) where both particles move with the same mean speed μ, and have the same diameter σ, and where the total number of particles per cubic unit is ν, the numbers of contacts per unit time between any one moving particle and the remaining $(\nu - 1)$ particles is,

$$N_b = \frac{\frac{4}{3} \mu \pi (\nu - 1) \sigma^2}{V - \frac{2}{3} (\nu - 1) \sigma^3} . \tag{2}$$

Under case (c), where the two types of particles have mean speeds μ_1 and μ_2, mean diameters of σ_1 and σ_2, and where the number of one type is ν, the number of contacts in unit time between a single particle of the other type and the ν particles is,

$$N_c = \frac{\frac{1}{6 \mu_1 \mu_2} \left[-(\mu_1 - \mu_2)^3 + (\mu_1 + \mu_2)^3 \right] \pi \nu \left(\frac{\sigma_1 + \sigma_2}{2} \right)^2}{V - \frac{4}{3} \pi \nu \left(\frac{\sigma_1 + \sigma_2}{2} \right)^3} . \tag{3}$$

The value of $r=\frac{1}{6\mu_1\mu_2}\left[-(\mu_1-\mu_2)^3+(\mu_1+\mu_2)^3\right]$ depends on whether $\mu_1>\mu_2$ or $\mu_2>\mu_1$ for if $\mu_1>\mu_2$ then,

$$r=\frac{3\mu_1^2+\mu_2^2}{3\mu_1},$$

and if $\mu_2>\mu_1$ then,

$$r=\frac{3\mu_2^2+\mu_1}{3\mu_2^2}.$$

This difficulty may be overcome by writing

$$r=\frac{1}{6\mu_1\mu_2}\left[-(|\mu_1-\mu_2|)^3+(\mu_1+\mu_2)^3\right]=F(\mu_1,\mu_2),$$

where the vertical lines around $\mu_1-\mu_2$ have their usual significance, meaning the *absolute value* of $\mu_1-\mu_2$.

In the case of contacts between individuals of the various life-history stages of *T. confusum*, it will be apparent that larva-egg, larva-pupa, adult-egg and adult-pupa contacts come under case (a); that larva-larva (where both larvae are of the same age) and adult-adult contacts come under case (b); and that larva-larva (where the two larvae are of different ages), and adult-larva contacts come under case (c).

Since, moreover, the volume $V-\frac{4}{3}\pi\nu\left(\frac{\sigma_1+\sigma_2}{2}\right)^2$ is merely the volume, G, of the flour, the above formulas reduce to

$$N_a=\frac{\mu\pi\left(\frac{\sigma_1+\sigma_2}{2}\right)^2}{G}, \tag{5}$$

$$N_b=\frac{\frac{4}{3}\mu\pi(\nu-1)\sigma^2}{G}, \tag{6}$$

$$N_c=\frac{F(\mu_1,\mu_2)\,\pi\nu\left(\frac{\sigma_1+\sigma_2}{2}\right)^2}{G}. \tag{7}$$

It is necessary at this point to discuss more fully the exact significance of σ_1 and σ_2 in the case of insects. It is not necessary that an insect come into actual physical contact with the main body wall of another insect to be aware of its presence. It may for example touch against a minute projecting bristle. As, then, a 'contact' will be defined as any approach of two entities of the population within such a distance of each other that mutual recognition of each other's presence occurs (or recognition by one party occurs, if the other be an egg or a pupa), it will be necessary to define what may be called "radii of perception".

It has been suggested to the writer that since the insects under discussion are assumed to have no intelligence, the term radius of *perception* is somewhat unfortunate. It is felt, however, that such is not the case, since by "intelligence" is meant, not the mere possession of powers of perception through the senses, but rather the possession of mental powers sufficient to make conscious and more or less elaborate decisions under definite conditions.

The radius of perception of a living entity for an infinitesimally small point is defined, then, as equal to the radius of a sphere whose volume V is

$$V = \int_{\alpha}^{\beta} \int_{Y_0}^{Y_1} \int_{Z_0}^{Z_1} J(X, Y, Z)dx\, dy\, dz, \tag{4}$$

where $J(X, Y, Z)$ is a surface surrounding the entity in space, beyond which its powers of perception are zero.

However, neither party in an insect contact such as have been discussed above has an infinitesimal diameter. Hence, where, for example, the radius of perception of an adult for an infinitesimal point is $\bar{r}_{11.p}$ and the radius of perception of a larva of some certain instar such that the correct subscript may in a general way be written as i, for the point is, $\bar{r}_{i.p}$, the radius of perception of either the larva for the adult, or *vice versa* is

$$r_{11.i} = r_{i.11} = \frac{\bar{r}_{11.p} + \bar{r}_{i.p}}{2} \text{ etc.}$$

In the case of contacts with eggs, the latter of course are incapable of perceiving anything so that, strictly, $r_{2.i} = 0$. It is obvious however that the quantity to be used in such a case in place of $\bar{r}_{2.p}$ is the mean radius of the egg.

Then where $M_{11.2}$, $M_{L.L}$ and $M_{11.L}$ are the numbers of contacts in unit time between adults and eggs, between generalized larvae of the same age, and between adults and generalized larvae respectively, and where μ_{11} and μ_L are the mean speeds of adults and generalized larvae,

$$\frac{dM_{11.2}}{dt} = \frac{N_{11}N_2 u_{11} \pi r_{11.2}^2}{G} \tag{8}$$

$$\frac{dM_{L.L}}{dt} = \frac{4N_L(N_L - 1)\mu_{11}\, \pi r_{L.L}^2}{3G} \tag{9}$$

$$\frac{dM_{11.L}}{dt} = \frac{N_{11}\, N_L \cdot F(\mu_{11}, \mu_L) \pi\, r_{11.L}^2}{G} \tag{10}$$

Amounts of Flour Encountered by Adults or Larvae

Since adults and larvae eat flour, as well as eggs, other larvae and pupae, it is necessary to develop functions descriptive of the rate at which flour is encountered by moving adults or larvae. Theoretically this function could be developed in a manner similar to the above-mentioned formulas for contacts between adults and eggs, etc., but a much simpler and neater derivation can be given by reason of the more or less continuous nature of the flour medium.

It might be felt that inasmuch as an adult or larva is continually surrounded on all sides by flour, and as, by reason of its homogeneous nature, one portion of flour is indistinguishable from any other portion, the amount of flour brought to the attention of a moving insect in unit time should be independent of the mean speed of the insect. (It should be noted that in speaking of the senses of insects, no anthropomorphic concept is intended; the words are used simply because more suitable expressions are not available.)

The writer feels however that such is not the case, for he cannot help but think that (to use unfortunately an anthropomorphic analogy) a steady stream of material passing across the field of perception gives an impression of volume proportional to the distance travelled by the stream in unit time.

Consider an adult moving in a random way in a space, G, filled with flour. Clearly the adult does not bore out an exactly circular tunnel but, for ease of consideration, the tunnel will be considered as circular, and of such a radius ρ that $\pi\rho^2 = \int_C xdy$ where the expression on the right is the line integral taken in the positive direction around the boundary curve of a right section of the tunnel.

It is further assumed that the anterior surface of an adult cephalad of a certain plane of right section Q is defined by a function $\phi\ (x, y, z)$; that only the flour in a layer of thickness D over the surface $\phi\ (x, y, z)$ is perceptible to the adult as food, and that when, due to forward movement of the insect, a particle of flour passes to a position posterior to Q, it ceases to be perceptible as food. It is also assumed, for the moment, that flour is a perfect fluid, *i.e.*, frictionless and incompressible.

If now, the surface $\phi\ (x, y, z)$ be moved a distance dx along the longitudinal axis of the insect, a certain frustum of thickness dx will pass beyond Q and be lost to perception.

With the exception of infinitesimals of higher order, the volume of this frustum is:

$$\begin{aligned} dv &= \pi(\rho+D+\rho)(\rho+D-\rho)dx \\ &= \pi(2\rho D+D^2)dx. \end{aligned}$$

Whence, on advancing the surface $\phi\ (x, y, z)$ at a rate μ_{11}, there passes across the field of perception of an adult, in unit time,

$$V = \int_0^{\mu_{11}} \pi(2\rho D+D^2)dx = \pi(2\rho D+D^2)\mu_{11},$$

which is the volume of a cylindrical shell of thickness D surrounding the bore of the tunnel cut out in the flour.

Then, where,

$M_{11.1}$ = volume of flour encountered by a mature adult up to a time T.

$M_{L.1}$ = a similar function for the generalized larva.

And where, for the sake of uniformity, we write:

$$r_{11.1}^2 = 2\rho_{11}D_{11}+D_{11}^2 \qquad r_{L.1}^2 = 2\rho_L D_L+D_L^2$$

Calling $r_{11.1}$ and $r_{L.1}$ the radii of perception for flour, we have:

$$\frac{dM_{11.1}}{dt} = \pi r_{11.1}^2\mu_{11}, \quad \frac{dM_{L.1}}{dt} = \pi r_{L.1}^2\ \mu_L.$$

In a practical case, however, flour is not in any sense a perfect fluid, but this makes no difference as far as the speeds μ_{11} and μ_L are concerned, as these are the speeds maintained in spite of the resistance of the flour. The fact of compressibility must however be taken into account, and this may be done by means of an arbitrary factor of compression, to be determined by experiment

for each type of flour and for each life-history stage. These factors are then of the form, K_3, K_4, K_5, K_6, K_7, K_8, K_{10}, K_{11}, according to the established notation for subscripts.

Including the above factors, where K_L is the subscript for the generalized larva,

$$\frac{dM_{11.1}}{dt} = K_{11}\pi r^2_{11.1}\mu_{11}, \qquad \frac{dM_{L.1}}{dt} = K_L \pi r^2_{L.1}\mu_L. \tag{12}$$

A Transformation of the Above Equations

So far the equations descriptive of rates of contact have been developed in terms of individual eggs, larvae, etc., but in order to determine the rates at which these various food materials are consumed it will be necessary to transform the above equations into others descriptive of the rates at which assimilable nutritive materials are encountered.

It would be difficult or even impossible to determine the exact requirements of each life-history stage in terms of the various fats, proteins, vitamins, etc., and if the various stages were at certain times dependent entirely upon one food and at other times entirely upon another, it would be necessary to have exact knowledge of the materials obtainable from each food source. However, as this is not the case, it is possible, without grave error, to make the assumption that each food material supplies all of the substances necessary to maintain the various entities feeding on it. It may be the case that eggs, for example, are lacking in some particular chemical substance if they are to function as the sole food, but this need not cause concern, as the necessary compound is obtainable from some source as evidenced by the vigorous growth of the feeders. That is to say, it does not appear that, throughout all the available foods, any compound is scarce to the point of detriment to the population.

Suppose that A_1, A_2, A_3 etc., are the assimilable percentages of nutrient material in the various life-history stages, W_2, W_3, etc., the weights of individuals of the various stages, and W_1 the density of uncompressed flour. We obtain, then, as equations descriptive of the rates at which assimilable nutrient materials are encountered, the following:

$$\frac{dY_{11.2}}{dt} = \frac{A_2 W_2 N_{11} N_2 \mu_{11} \pi r^2_{11.2}}{G} \tag{13}$$

$$\frac{dY_{11.L}}{dt} = \frac{A_L W_L N_{11} N_L \cdot F(\mu_{11},\mu_L) \pi r^2_{11.L}}{G} \tag{14}$$

$$\frac{dY_{L.2}}{dt} = \frac{A_2 W_2 N_L N_2 \mu_L \pi r^2_{L.2}}{G} \tag{15}$$

$$\frac{dY_{11.1}}{dt} = \frac{A_1 W_1 N_{11} K_{11} \mu_{11} \pi r^2_{11.1} G}{G} = A_1 W_1 N_{11} K_{11} \mu_{11} \pi r^2_{11.1} \tag{16}$$

$$\frac{dY_{L.1}}{dt} = \frac{A_1 W_1 N_L K_L \mu_L \pi r^2_{L.1} G}{G} = A_L W_L N_L K_L \mu_L \pi r^2_{L.1}. \tag{17}$$

It is now possible to set up the differential equations for the early stages of the population growth, except for one point discussed below.

It will be apparent that (considering the insect from a purely mechanistic viewpoint) there is a certain definite quantity of nutrient material which must be assimilated in unit time to carry on the processes of life during that unit time. (The question as to what would occur in vitamin-deficient foods need not enter here as the use of whole-wheat flour obviates the possibility of such deficiencies.)

In actual practice it is not certain that the various life-history stages feed each one at a constant rate throughout the day. However, as they were kept in darkness, and the flour environment is at all times invariant, and there is at all times an abundance of food, it is assumed, lacking evidence to the contrary, that assimilable materials are ingested at such an average and approximately constant rate that the maintainance ration of the same is obtained for each increment of time. It is apparent that such an assumption cannot exactly describe what goes on, as, if larvae of an age α happen to be singularly lacking in assimilable materials, an adult, while consuming one of them, cannot, perhaps, maintain this assumed rate of ingestion. However, this is not a grave matter, as the above assumption means that, with the exception of negligible quantities, the required amount of material is ingested over a finite time, even though the rate of ingestion may be small at some one instant.

This assumed, approximately constant rate of ingestion will be referred to as the "maintainance rate of ingestion", with the additional assumption that the digestive powers of all life-history stages are the same. Symbols of the form E_3, E_4, etc. will be used to denote it.

The Population During the Period $t_0 = t_2(1) < T < t_3(1)$

During this period, mature adults are laying eggs and at the same time are finding and eating them, whence the rate of change of the egg population is equal to the difference between the rates of egg production and egg consumption. .

Let:

$C_{11.2}$ = number of individual eggs consumed by the N_{11} mature adults up to a time T.

$X_{11.2}$, $X_{11.1}$ = amounts of assimilable material, (by weight) obtained from eggs and flour respectively, and consumed by the N_{11} mature adults up to a time T.

$P_{11.2}$, $P_{11.1}$ = constant coefficients of preference of mature adults for eggs and flour respectively, as foods.

Then:

$$\frac{dN_2}{dt} = R\epsilon N_{11} - C'_{11.2} \tag{18}$$

From the definition of $P_{11.2}$ and $P_{11.1}$,

$$\frac{X'_{11.2}}{P_{11.2}\, Y'_{11.2}} = \frac{X'_{11.1}}{P_{11.1}\, Y'_{11.1}}. \tag{19}$$

From the definition of E_{11},

$$X'_{11.2}+X'_{11.1}=E_{11}N_{11}. \tag{20}$$

From (19)
$$X'_{11.1} = \frac{P_{11.1}Y'_{11.1}\cdot X'_{11.2}}{P_{11.2}Y'_{11.2}}. \tag{21}$$

Substituting in (20)

$$X'_{11.2} + \frac{P_{11.1}Y'_{11.1}\cdot X'_{11.2}}{P_{11.2}Y'_{11.2}} = E_{11}N_{11}, \tag{21}$$

$$X'_{11.2}=\frac{E_{11}N_{11}P_{11.2}Y'_{11.2}}{P_{11.2}Y'_{11.2}+P_{11.1}Y'_{11.1}}. \tag{22}$$

$$X'_{11.2}=\frac{\dfrac{E_{11}N_{11}P_{11.2}A_2W_2\mu_{11}\pi r^2_{11.2}N^2_{11}N_2}{G}}{\dfrac{P_{11.2}A_2W_2\mu_{11}\pi r^2_{11.2}N_{11}N_2}{G}+\dfrac{P_{11.1}A_1W_1K_{11}\mu_{11}\pi r^2_{11.1}GN_{11}}{G}}, \tag{23}$$

$$C'_{11.2}=\frac{X'_{11.2}}{A_2W_2}=\frac{E_{11}P_{11.2}r^2_{11.2}N_{11}N_2}{P_{11.2}A_2W_2r^2_{11.2}N_2+P_{11.1}A_1W_1K_{11}r^2_{11.1}G}, \tag{24}$$

$$C'_{11.2}=\frac{HN_2}{cN_2+d}, \tag{25}$$

Where $H=E_{11}\,P_{11.2}\,r^2_{11.2}\,N_{11}$; $c=P_{11.2}\,A_2\,W_2\,r^2_{11.2}$; $d=P_{11.1}\,A_1\,W_1\,K_{11}\,r^2_{11.1}\,G$.

Whence

$$\frac{dN_2}{dt}=\frac{R\epsilon N_{11}cN_2+R\epsilon N_{11}d-HN_2}{cN_2+d}. \tag{26}$$

$$\frac{dN_2}{dt}=\frac{aN_2+b}{cN_2+d} \tag{27}$$

Where $a=R\epsilon N_{11}c-H$; $b=R\epsilon N_{11}d$.

Separating the variables and integrating:

$$\frac{cN_2}{a}+\frac{ad-bc}{a^2}\log(aN_2+b)=t+c_1 \tag{28}$$

When $t=0,\ N_2=0,$

So that
$$c_1=\frac{ad-bc}{a^2}\log\ b,$$

Whence
$$T=\frac{c}{a}N_2+\frac{ad-bc}{a^2}\log\left(\frac{aN_2+b}{b}\right)=F(N_2). \tag{29}$$

Characteristics of the function $T=F(N_2)$, (See Fig. 1).

We may write,

$$aN_2+b=(R\epsilon N_{11}c+H)N_2+R\epsilon N_{11}d$$

$$aN_2+b=N_{11}P_{11.2}r^2_{11.2}\left[R\epsilon A_2W_2-E\right]N_2+R\epsilon N_{11}P_{11.1}A_1W_1K_{11}r^2_{11.1}G,$$

from which it is evident that a is less than zero in any real population, since $R\epsilon A_2W_2$, the amount of assimilable nutrient material expended per female per day in the form of eggs, must be less than E_{11}, the total amount of assimilable nutrient material taken in per female per day, *i.e.*, $(R\epsilon A_2W_2 - E_{11}) < 0$.

Hence, as b, c and d are easily seen to be greater than zero,

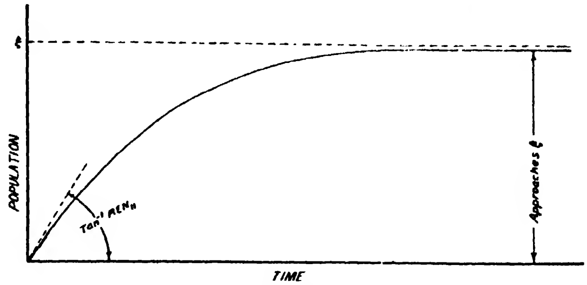

FIG. 1. *Graph of the function,* $T = F(N_2)$.

$$\frac{dN_2}{dt} \gtreqless 0 \quad \text{as} \quad N_2 \lesseqgtr -\frac{b}{a} = \xi \,. \tag{30}$$

It can further be shown that $T = F(N_2)$ may be written as a convergent alternating power series of the form,

$$N_2 = \alpha_1 T - \alpha_2 T^2 + \alpha_3 T^3 - \alpha_4 T^4 + \ldots \ldots , \tag{31}$$

and that

$$\alpha_1 = R\epsilon N_{11} \;,$$

whence

$$\lim_{T \to 0} \frac{dN_2}{dt} = R\epsilon N_{11} \;.$$

Also, since $\dfrac{dN_2}{dt} = \dfrac{aN_2+b}{cN_2+d}$, $\displaystyle\lim_{N_2 \to 0} \frac{dN_2}{dt} = \frac{b}{d} = R\epsilon N_{11} = \lim_{T \to 0} \frac{dN_2}{dt}$.

It is also evident from an examination of (31) that $\displaystyle\lim_{T \to 0} N_2 = 0$ and it can further be shown by direct differentiation that $\displaystyle\lim_{N_2 \to \xi} \frac{d^2N_2}{dT^2} = 0$

It is thus apparent that the egg population is equal to zero when T is equal to zero, and increases thereafter to approach a value ξ which it reaches only after an infinite time. (In an actual case, owing to the fact that the function $T = F(N_2)$ can exist only for integral values of N_2, this limit, ξ, may be reached in finite time, the population oscillating around the value ξ, and between the limits of the least integer greater than ξ, and the greatest integer less than ξ.)

Furthermore, when $T = T_0$, the egg population is increasing exactly at the rate at which the N_{11} mature adults lay eggs, namely, at the rate $R\epsilon N_{11}$. This rate decreases thereafter, reaching zero when N_2 equals ξ.

It is interesting to note the meaning of ξ. It represents the point at which the eggs are found and eaten as rapidly as laid. Thus, if eggs are introduced into the system by artificial means, as by adding them to the flour by hand, and stirring them in, they will be found and eaten more rapidly than the N_{11}

adults can lay, so that $\frac{dN_2}{dT} < 0$ and the egg population decreases to approach the limit ξ from above. Such conditions as have been described above occur only, of course, in the absence of hatching.

It is also of interest to examine the relationship between ξ and G, the size of the flour mass. Since hatching does not occur, N_{11} is a constant.

We may write, $b = R\epsilon N_{11}d = R\epsilon N_{11}P_{11.1}A_1W_1K_{11}r_{11.1}^2G = \gamma G$,

whence
$$\xi = \frac{\gamma G}{a},$$

and
$$\frac{d\xi}{dG} = \frac{\gamma}{a} = \text{a constant}. \quad (32)$$

Whence, the final egg population (in the absence of hatching), is proportional to the size of the environment. Since we may further write, $a = \theta N_{11}$,

where
$$\theta = P_{11.2}A_2W_2r_{11.2}^2(R\epsilon A_2W_2 - E_{11}) .$$
and
$$b = \phi N_{11} ,$$
where
$$\phi = R\epsilon A_1W_1K_{11}r_{11.1}^2G .$$
Then
$$\xi = \frac{\phi N_{11}}{\theta N_{11}} = \frac{\phi}{\theta}. \quad (33)$$

which is independent of the value of N_{11}.

Chapman (7) has shown that the limiting population reached with *T. confusum* is, by actual experiment, proportional to the size of the environment, and independent of the initial number of adults. It is true that at any temperature above 17°C. hatching occurs, so that the final population is a mixture

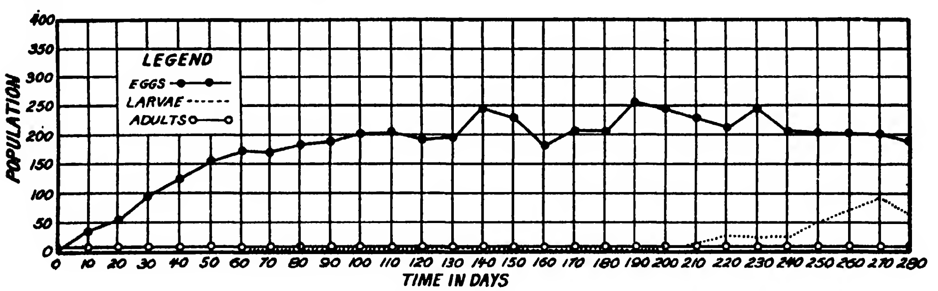

FIG. 2. *Population growth of T. confusum, Duv. at 17°C., mean of (A) and (B) populations.*

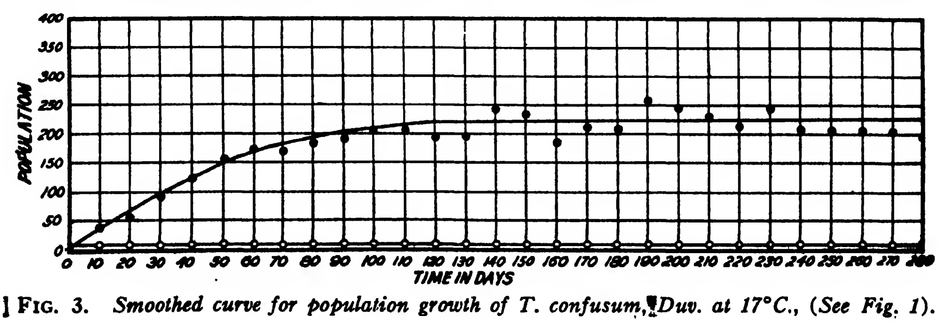

FIG. 3. *Smoothed curve for population growth of T. confusum, Duv. at 17°C., (See Fig. 1).*

of all stages, but it can at least be said that theory and experiment are in substantial agreement up to 17°C. Further elaboration of the theory will, it is believed, show similar agreement at higher temperatures.

At 17°C. the rate of hatching is so low that it can be neglected, and, as Figs. 2 and 3 show, the population curve follows very closely the theoretical curve of Fig. 1. It should be noted that the theoretical curve of Fig. 3 was not drawn from values computed from Equation (29), as, owing to lack of knowledge of several biotic constants, it is impossible to obtain such numerical solutions. It was however drawn on a basis of the information obtained as to its shape, from Equation (29). The claim to agreement is thus on the form of the curve only.

The Population Growth Subsequent to $T=t_3(1)$

Subsequent to $T=t_3(1)$, *i.e.*, after the first hatching of eggs commences, the problem assumes much greater complexity, due not only to the presence of larvae which act as predators, but also owing to the complicated functions descriptive of the rates of transformation of each life-history stage into the succeeding one.

The general form of the equations

By an extension of the reasoning used to determine Equation 18, it will be seen that the rate of change of the egg population is equal to the basic rate of laying of eggs, namely, $R\epsilon N_{11}$, minus the rate at which eggs as individuals are being eaten by the adults $(C'_{10.2}+C'_{11.2})$, minus the rate at which eggs are being eaten by the various larvae of different instars $(C'_{3.2}, C'_{4.2}, \ldots . C'_{8.2})$, minus the rate at which eggs are arriving at hatching age (Z_3). It should be noted that not all eggs which arrive at hatching age actually hatch. A certain percentage shrivel up and die at this time. This matter will be referred to later.

Thus, in a purely general way, where all six larval instars are present

$$\frac{dN_2}{dt} = R\epsilon N_{11} - \sum_{i=3}^{i=8} C'_{i\cdot 2} - C'_{10.2} - C'_{11.2} - Z_3 \quad . \tag{34}$$

Certain assumptions are necessary in order that the differential equations descriptive of the growths of larval populations may be written. Some changes do occur in the biotic characteristics of a larva of any given instar during the time that it spends in that instar but, as these changes are relatively small, it will be assumed that the various parameters descriptive of each instar are constant throughout the life of that instar, and that the change from instar to instar is abrupt and discontinuous.

It has been stated that not all eggs arriving at hatching age actually hatch. The same thing applies to all subsequent transformations. Hence if U_3, $U_4 \ldots .$ U_{11} be the percentages of deaths at egg-first instar, first instar-second instar, etc., transformations, then, when a form $(i-1)$ is arriving at the age of transformation to a form i at a rate Z_i, the form i is coming into existence at a rate U_iZ_i.

Then if N_j be the number of larvae of the form having subscript j at any time T, and $C'_{i.j}$ be the rate at which they are being consumed by other larvae of forms having the general subscript i, etc., then, in a purely general way,

$$\frac{dN_j}{dt} = U_j Z_j - \sum_{i=3}^{i=8} C'_{i.j} - C'_{10.j} - C'_{11.j} - Z_{j+1}\,. \qquad (35)$$

It will be seen later that some or all of the C''s may be zero, depending upon the circumstances at a particular time.

It is apparent that an equation similar to that descriptive of the growth of the egg population can be written in the case of the growth of the pupal population.

Evaluation of the C''s

It is assumed, as again is practically true, except in the case of some of the smaller larvae, that no larva of a given instar can consume one of the succeeding instar, and that adults are immune from attack by any form, even by other

TABLE VIII

$\frac{X'_{11.1}}{Q_{11.1}}$	$\frac{X'_{11.2}}{Q_{11.2}}$	$\frac{X'_{11.3}}{Q_{11.3}}$	$\frac{X'_{11.4}}{Q_{11.4}}$	$\frac{X'_{11.5}}{Q_{11.5}}$	$\frac{X'_{11.6}}{Q_{11.6}}$	$\frac{X'_{11.7}}{Q_{11.7}}$	$\frac{X'_{11.8}}{Q_{11.8}}$	$\frac{X'_{11.9}}{Q_{11.9}}$	1	1
$\frac{X'_{10.1}}{Q_{10.1}}$	$\frac{X'_{10.2}}{Q_{10.2}}$	$\frac{X'_{10.3}}{Q_{10.3}}$	$\frac{X'_{10.4}}{Q_{10.4}}$	$\frac{X'_{10.5}}{Q_{10.5}}$	$\frac{X'_{10.6}}{Q_{10.6}}$	$\frac{X'_{10.7}}{Q_{10.7}}$	$\frac{X'_{10.8}}{Q_{10.8}}$	$\frac{X'_{10.9}}{Q_{10.9}}$	1	1
1	1	1	1	1	1	1	1	1	1	1
$\frac{X'_{8.1}}{Q_{8.1}}$	$\frac{X'_{8.2}}{Q_{8.2}}$	$\frac{X'_{8.3}}{Q_{8.3}}$	$\frac{X'_{8.4}}{Q_{8.4}}$	$\frac{X'_{8.5}}{Q_{8.5}}$	$\frac{X'_{8.6}}{Q_{8.6}}$	$\frac{X'_{8.7}}{Q_{8.7}}$	$\frac{X'_{8.8}}{Q_{8.8}}$	$\frac{X'_{8.9}}{Q_{8.9}}$	1	1
$\frac{X'_{7.1}}{Q_{7.1}}$	$\frac{X'_{7.2}}{Q_{7.2}}$	$\frac{X'_{7.3}}{Q_{7.3}}$	$\frac{X'_{7.4}}{Q_{7.4}}$	$\frac{X'_{7.5}}{Q_{7.5}}$	$\frac{X'_{7.6}}{Q_{7.6}}$	$\frac{X'_{7.7}}{Q_{7.7}}$	1	$\frac{X'_{7.9}}{Q_{7.9}}$	1	1
$\frac{X'_{6.1}}{Q_{6.1}}$	$\frac{X'_{6.2}}{Q_{6.2}}$	$\frac{X'_{6.3}}{Q_{6.3}}$	$\frac{X'_{6.4}}{Q_{6.4}}$	$\frac{X'_{6.5}}{Q_{6.5}}$	$\frac{X'_{6.6}}{Q_{6.6}}$	1	1	$\frac{X'_{6.9}}{Q_{6.9}}$	1	1
$\frac{X'_{5.1}}{Q_{5.1}}$	$\frac{X'_{5.2}}{Q_{5.2}}$	$\frac{X'_{5.3}}{Q_{5.3}}$	$\frac{X'_{5.4}}{Q_{5.4}}$	$\frac{X'_{5.5}}{Q_{5.5}}$	1	1	1	$\frac{X'_{5.9}{}^{*}}{Q_{5.9}}$	1	1
$\frac{X'_{4.1}}{Q_{4.1}}$	$\frac{X'_{4.2}}{Q_{4.2}}$	$\frac{X'_{4.3}}{Q_{4.3}}$	$\frac{X'_{4.4}}{Q_{4.4}}$	1	1	1	1	$\frac{X'_{4.9}{}^{*}}{Q_{4.9}}$	1	1
$\frac{X'_{3.1}}{Q_{3.1}}$	$\frac{X'_{3.2}}{Q_{3.2}}$	$\frac{X'_{3.3}}{Q_{3.3}}$	1	1	1	1	1	$\frac{X'_{3.9}{}^{*}}{Q_{3.9}}$	1	1
1	1	1	1	1	1	1	1	1	1	1
1	1	1	1	1	1	1	1	1	1	1

* *It seems doubtful if these are of much importance since $P_{5.9}$, $P_{4.9}$ and $P_{3.9}$ are small and probably zero.*

adults. (This latter assumption is strictly true, except for a very brief period during which a new adult is emerging from the pupal skin.) These assumptions may be introduced into the formulas by writing $P_{i.j}=0$, where $j>i$, (except $j=9$), and $P_{i.10}$, $P_{i.11}=0$ for all values of i. It is also evident that, of necessity, as flour, eggs and pupae cannot eat, all P's of the forms $P_{1.j}$, $P_{2.j}$ and $P_{9.j}$ are zero.

The C''s may now be evaluated by an extension of the reasoning used in Equations 19 to 24. As such symbols as $\frac{X'_{i.j}}{P_{i.j}Y'_{i.j}}$ occur frequently, they will be written as $\frac{X'_{i.j}}{Q_{i.j}}$.

Certain of these are of no importance, namely, those for which $P_{i.j}=0$. Such a case gives, of course, the indeterminate form $\frac{0}{0}$, but it is easy to see that this reduces to the value 1. There result, then, the ratios shown in Table VIII, all equal. Those for which $P_{i.j}=0$ have been written as 1 immediately, and thus disposed of.

As, (See Equation 20)

$$E_iN_i = \sum_{j=1}^{j=11*} X'_{i.j},$$

and as any one of the X's may be obtained in terms of any other in the same row by means of the relationship

$$X'_{i.j} = \frac{X'_{i.k}Q_{i.j}}{Q_{i.k}}, \tag{36}$$

on substitution,

$$E_iN_i = \frac{X'_{i.k}}{Q_{i.k}} \sum_{j=1}^{j=11} Q_{i.j}, \tag{37}$$

whence

$$X'_{i.k} = \frac{E_iN_iQ_{i.k}}{\sum_{j=1}^{j=11} Q_{i.j}}, \tag{38}$$

which, on dividing by W_kA_k, becomes

$$C'_{i.k} = \frac{E_iN_iQ_{i.k}}{W_kA_k \sum_{j=1}^{j=11} Q_{i.j}}. \tag{39}$$

It is now necessary to consider the forms of the Q's. Some are equal to zero directly, and are thus of no further interest. These are Q's of the forms $Q_{1.j}$, $Q_{2.j}$, $Q_{9.j}$, $Q_{i.j}$ where $i<j$ (except $j=9$), and $Q_{i.10}$, $Q_{i.11}$ for all values of i. It must be remembered, of course, that any $Q_{i.j}$ is zero if N_i is zero.

As a preliminary step, the Q's may be divided into four classes, (a), (b) and (c) as on page 642, with an additional class (a_1) for encounters with flour. Thus remembering that $Q_{i.j}=P_{i.j}\ Y'_{i.j}$

*The summation may be made from 1 to 11 since $X'_{i.11}$ and $X'_{i.10}=0$.

Under case (a_1),

$$Q_{3\,1} = \frac{P_{3\,1}A_1W_1K_3\mu_3\pi r_{3\,1}^2N_3G}{G}, \tag{40}$$

$$Q_{i\,1} = \frac{P_{i\,1}A_1W_1K_i\mu_i\pi r_{i\,1}^2N_iG}{G}, \tag{41}$$

$$\vdots$$

$$Q_{11\,1} = \frac{P_{11\,1}A_1W_1K_{11}\mu_{11}\pi r_{11\,1}^2N_{11}G}{G}. \tag{42}$$

Under case (a) there are formulas descriptive of amounts of assimilable material obtained by larvae or adults from eggs or pupae, thus

$$Q_{3\,2} = \frac{P_{3\,2}A_2W_2N_3N_2\mu_3\pi r_{3\,2}^2}{G}, \tag{43}$$

$$Q_{4\,2} = \frac{P_{4\,2}A_2W_2N_4N_2\mu_4\pi r_{4\,2}^2}{G}, \tag{44}$$

$$Q_{10\,2} = \frac{P_{10\,2}A_2W_2N_{10}N_2\mu_{10}\pi r_{10\,2}^2}{G}, \tag{45}$$

$$Q_{6\,9} = \frac{P_{6\,9}A_9W_9N_6N_9\mu_6\pi r_{6\,9}^2}{G}, \tag{46}$$

$$Q_{11\,9} = \frac{P_{11\,9}A_9W_9N_{11}N_9\mu_{11}\pi r_{11\,9}^2}{G}. \tag{47}$$

Under case (b) there are formulas descriptive of amounts of assimilable material obtained from larvae of a given age by larvae of the same age, thus:

$$Q_{3\,3} = \frac{4P_{3\,3}A_3W_3N_3(N_3-1)\mu_3\pi r_{3\,3}^2}{3G}, \tag{48}$$

$$Q_{4\,4} = \frac{4P_{4\,4}A_4W_4N_4(N_4-1)\mu_4\pi r_{4\,4}^2}{3G}, \tag{49}$$

$$\vdots$$

$$Q_{i\,i} = \frac{4P_{i\,i}A_iW_iN_i(N_i-1)\mu_i\pi r_{i\,i}^2}{3G}, \tag{50}$$

$$\vdots$$

$$Q_{8\,8} = \frac{4P_{8\,8}A_8W_8N_8(N_8-1)\mu_8\pi r_{8\,8}^2}{3G}. \tag{51}$$

Under case (c) there are formulas descriptive of amounts of assimilable materials obtained from larvae of various ages, by larvae not of the same ages, and by adults, either mature or immature, thus:

$$Q_{4.3} = \frac{P_{4.3}A_3W_3N_4N_3F(\mu_4, \mu_3)\pi r_{4.3}^2}{G}, \tag{52}$$

$$Q_{5.3} = \frac{P_{5.3}A_3W_3N_5N_3F(\mu_5, \mu_3)\pi r_{5.3}^2}{G}, \tag{53}$$

$$Q_{11.6} = \frac{P_{11.6}A_6W_6N_{11}N_6F(\mu_{11}, \mu_6)\pi r_{11.6}^2}{G}. \tag{54}$$

The C''s may now be evaluated in terms of the Q's. The formulations may be simplified as a result of an examination of $F(\mu_i, \mu_j)$. It has been convenient hitherto to use the separate velocity corrections; it will now be shown that they may all be written in the form $F(\mu_i, \mu_j)$.

$$F(\mu_i, u_j) = \frac{1}{6\mu_i\mu_j}\left[-(|\mu_i - \mu_j|)^3 + (\mu_i + u_j)^3\right]. \tag{55}$$

Suppose that $\mu_i > \mu_j$

Then if μ_i or μ_j is zero, while the other is not, clearly it must be $\mu_j = 0$.

Whence
$$F(\mu_i, \mu_j) = \frac{3\mu_i^2\mu_j - \mu_j^3}{3\mu_i\mu_j}. \tag{56}$$

Differentiating both numerator and denominator separately with respect to μ_j we obtain,

$$\frac{3\mu_i^2 + 3\mu_j^2}{3\mu_i^2}.$$

Whence
$$\lim_{\mu_j \to 0} F(\mu_i, \mu_j) = \mu_i.$$

Similarly it can be shown that where $\mu_j > \mu_i$,

$$\lim_{\mu_i \to 0} F(\mu_i, \mu_j) = \mu_j.$$

It is also evident from direct substitution in (55) that,

$$\lim_{\mu_i \to \mu_j} F(\mu_i, \mu_j) = \frac{4}{3}\mu_i \text{ (or } \frac{4}{3}\mu_j).$$

Whence, where $i = 3, 4, 5, \ldots .8, 10$ and 11,

$$C'_{i.1} = \frac{\dfrac{P_{i.1}W_1A_1K_iF(\mu_i,0)\pi r_{i.1}^2N_i^2G\,E_i}{G}}{W_1A_1\left[\dfrac{P_{i.1}W_1A_1K_iF(\mu_i,0)\pi r_{i.1}^2N_iG}{G} + \displaystyle\sum_{j=2}^{j=9}\dfrac{P_{i.j}W_jA_jF(\mu_i,u_j)\pi r_{i.j}^2N_iN_j}{G}\right]}, \tag{57}$$

$$C'_{i.1} = \frac{P_{i.1}K_iF(\mu_i, 0)\, r^2_{i.1}\, E_iN_iG}{P_{i.1}W_1A_1K_iF(\mu_i, 0)r^2_{i.1}G + \sum_{j=2}^{j=9} P_{i.j}W_jA_j\, F(\mu_i, \mu_j)r^2_{i.j}N_j} \tag{58}$$

and where $j = 2, 3, 4, \ldots .9$ and $j \neq i$

$$C'_{i.j} = \frac{P_{i.j}F(\mu_i, \mu_j)r^2_{i.j}N_iN_jE_i}{P_{i.1}W_1A_1K_iF(\mu_i, 0)\, r^2_{i.1}G + \sum_{k=2}^{k=9} P_{i.k}W_kA_k\, F(\mu_i, \mu_k)r^2_{i.k}N_k} \tag{59}$$

and where $j = i = 3, 4, 5, 6, 7, 8$, ($C'_{i.j} = 0$ when $i = 1, 2, 9$, or $j = 10$ or 11)

$$C'_{i.i} = \frac{P_{i.i}F(\mu_i, \mu_i)r^2_{i.i}\, N_i\, (N_i - 1)E_i}{P_{i.1}W_1A_1F(\mu_i, 0)r^2_{i.1}\, G + \sum_{k=2}^{k=9} P_{i.k}W_kA_k\, F(\mu_i, \mu_k)r^2_{i.k}N_k} \tag{60}$$

from which it is readily seen that $C'_{i.j}$ is zero if either N_i or N_j is zero.

Determination of the forms of the Z's

As stated above, Z_i is the rate at which a form with subscript $(i-1)$ is transforming to a form (i) at a time T.

Since the Z's involve certain functions the forms of which have not yet been determined, only a cursory explanation of the forms of the Z's will be given here, a more detailed explanation being reserved for later publication.

By an extension of notation previously given*, let t_2, $t_3 \ldots\ldots t_{11}$ be the fixed times at which transformation to the forms having subscripts $2, 3, 4, \ldots\ldots 11$ commences, *i.e.*, the first pupa comes into existence at t_9. Also let $\Gamma_2, \Gamma_3, \ldots\ldots \Gamma_{10}$ be the durations of individuals in the various life-history stages having subscripts from 2 to 10.†

It will be clear that the history of an adult, for example, reaching maturity at a time T may be summarized as follows: laid as an egg at $T - \sum_2^{10} \Gamma_i$; hatched to a first instar larva at $T - \sum_3^{10} \Gamma_i$; transformed to a second, third, fourth, fifth and sixth instar larva at the times, $T - \sum_4^{10} \Gamma_i$, $T - \sum_5^{10} \Gamma_i$, $T - \sum_6^{10} \Gamma_i$, $T - \sum_7^{10} \Gamma_i$, $T - \sum_8^{10} \Gamma_i$ respectively; pupated at $T - \Gamma_{10} - \Gamma_9$; emerged as an immature adult at $T - \Gamma_{10}$; and became mature at T. Thus with each individual there are associated past times at which its various transformations took place.

Consider two groups of eggs, α and β, one of which, α, hatches promptly at t_3, while the other hatches later. In the case of the first group, α, the depredations due to eating have been caused only by the N_{11} adults originally present at $T = t_2 = 0$, whereas in the case of the group β, in the early part of its

**As only first generation eggs will be discussed, we may write t_2, t_3, t_4, rather than $t_2(1)$ $t_2(2)$ etc.*
†*Γ_{11} is indefinite, being the life of the mature adult, as such.*

life as eggs it was attacked only by the N_{11} mature adults, while in the later part of its life it was attacked by both the N_{11} mature adults and the N_3 first instar larvae existing subsequent to the time t_3. It is thus apparent that any function embodying the rate at which this group, β, has been eaten at various times, must be discontinuous at t_3. If moreover, T be greater than t_{11}, discontinuities must also exist at such of the times $t_2, t_3, \ldots\ldots t_{11}$ as fall within the time span over which the group has been in existence as a group of eggs. Similar discontinuities will occur in other functions embodying the rates of eating of other forms, and since the Z's are such functions, the above discontinuities will occur in the Z's as will be shown below. For reference the values of t_2 to t_{11} and of Γ_2 to Γ_{10} are given in Table IX, computed from Tables I and V.

TABLE IX

VALUES OF t_2 TO t_{11} AND Γ_2 TO Γ_{10} AT 27° C.

Γ	Value	t	Value	Derivation of t
Γ_2	6.04	t_2	0.00	$t_2=t_0=0$
Γ_3	2.43	t_3	6.04	$t_2+\Gamma_2$
Γ_4	3.63	t_4	8 47	$t_2+\Gamma_2+\Gamma_3$
Γ_5	3.03	t_5	12.10	$t_2+\Gamma_2+\ldots\ldots\Gamma_4$
Γ_6	3.27	t_6	15.13	$t_2+\Gamma_2+\ldots\ldots\Gamma_5$
Γ_7	3.39	t_7	18.40	$t_2+\Gamma_2+\ldots\ldots\Gamma_6$
Γ_8	6.67	t_8	21.74	$t_2+\Gamma_2+\ldots\ldots\Gamma_7$
Γ_9	8.64	t_9	28.46	$t_2+\Gamma_2+\ldots\ldots\Gamma_8$
Γ_{10}	6.04	t_{10}	37 10	$t_2+\Gamma_2+\ldots\ldots\Gamma_9$
		t_{11}	43.14	$t_2+\Gamma_2+\ldots\ldots\Gamma_{10}$

It has been shown (Equation 31) that

$$\lim_{T-t_2=0}\frac{dN_2}{dT}=R\epsilon N_{11},$$

that is, eggs are coming into existence at this rate when $T=t_2=0$.

Consider the rate at which eggs are arriving at hatching age when $T=t_3$. Clearly this must be less than $R\epsilon N_{11}$ since many eggs have been eaten.

Let this rate be $\theta(t_3)$.

Then the total reduction from the rate at which eggs were coming into existence at $T=t_2=0$ to the rate at which they are arriving at hatching age at t_3 is $R\epsilon N_{11}-\theta(t_3)$.

But the rate of eating of a small group of eggs laid between $T=t$ and $T=t+dt$ is, at any time t,

$$\frac{\theta(t)C'_{11.2}}{N_2}\,dt,$$

whence

$$\theta(t_3)=R\epsilon N_{11}-\int_{t_2}^{t_3}\frac{\theta(t)C'_{11.2}}{N_2}\,dt. \tag{61}$$

When $T=t_3$, first instar larvae (subscript 3) are present, whence during a sufficiently small neighborhood where $T>t_3$

$$\theta(T)=R\epsilon N_{11}-\int_{T-\Gamma_2}^{t_3}\frac{\theta(t)C'_{11.2}}{N_2}\,dt-\int_{t_3}^{T}\frac{\theta(t)\left(C'_{11.2}+C'_{3.2}\right)}{N_2}\,dt. \tag{62}$$

From Table IX it will be seen that $t_3=6.04$ and that $t_4=8.47$, whence, as $\Gamma_2=6.04$ and $\Gamma_3=2.43$, it is possible for T to exceed t_4 without $T-\Gamma_2$

exceeding t_3. In such a case, fourth instar larvae are present during the period from t_4 to T and we have

$$\theta(T)=R\epsilon N_{11}-\int_{T-\Gamma_2}^{t_3}\frac{\theta(t)C'_{11.2}}{N_2}\,dt-\int_{t_3}^{t_4}\frac{\theta(t)\left(C'_{11.2}+C'_{3.2}\right)}{N_2}\,dt$$
$$-\int_{t_4}^{T}\frac{\theta(t)\left(C'_{11.2}+C'_{4.2}+C'_{3.2}\right)}{N_2}\,dt. \tag{63}$$

When $T=12.08$, $T-\Gamma_2=t_3$ and the first integral on the right hand of Equation 63 becomes zero, since the upper and lower limits are equal.

When T becomes greater than 12.08, $T-\Gamma_2$ becomes greater than t_3 so that the integral becomes negative. Since this has no connection with any real case in an actual population, the fact that only positive integrals are to be considered will be denoted by a plus sign, thus: $\overset{+}{\int}$.

As for a sufficiently small neighborhood, $T>12.08$

$$\theta(T)=R\epsilon N_{11}-\overset{+}{\int_{T-\Gamma_2}^{t_4}}\frac{\theta(t)\left(C'_{11.2}+C'_{3.2}\right)}{N_2}\,dt-\overset{+}{\int_{t_4}^{T}}\frac{\theta(t)\left(C'_{11.2}+C'_{4.2}+C'_{3.2}\right)}{N_2}\,dt. \tag{64}$$

It is seen that as T increases, there is a periodic addition of integrals on the right hand, and a concomitant extinction of integrals on the left, accompanied by a migration of the lower limit $T-\Gamma_2$. This migration will be denoted by an arrow, thus: $\overset{+}{\int}_{\underrightarrow{T-\Gamma_2}}$.

Whence, for a value of T greater than say t_i where t_i is any one of the t's

$$\theta(T)=R\epsilon N_{11}-\overset{+}{\int}_{\underrightarrow{T-\Gamma_2}}^{t_3}\frac{\theta(t)C'_{11.2}}{N_2}\,dt-\overset{+}{\int_{t_3}^{t_4}}\frac{\theta(t)\left(C'_{11.2}+C'_{3.2}\right)}{N_2}\,dt$$
$$-\overset{+}{\int_{t_4}^{t_5}}\frac{\theta(t)\left(C'_{11.2}+\sum_{j=3}^{j=4}C'_{j.2}\right)}{N_2}\,dt-\cdots\cdots\overset{+}{\int_{t_i}^{T}}\frac{\theta(t)\left(C'_{11.2}+\sum_{j=3}^{j=i}C'_{j.2}\right)}{N_2}\,dt, \tag{65}$$

which may be written in contracted form as

$$\theta(T)=R\epsilon N_{11}-\overset{+}{\int}_{\underrightarrow{T-\Gamma_2}}^{t_3}\frac{\theta(t)\,C'_{11.2}}{N_2}\,dt-\sum_{k=3}^{k=i-1}\overset{+}{\int_{t_k}^{t_{k+1}}}\frac{\theta(t)\left(C'_{11.2}+\sum_{j=3}^{j=k}C'_{j.2}\right)}{N_2}\,dt$$
$$-\overset{+}{\int_{t}^{T}}\frac{\theta(t)\left(C'_{11.2}+\sum_{j=3}^{j=i}C'_{j.2}\right)}{N_2}\,dt. \tag{66}$$

It will be apparent that similar functions may be written for the rates of transformation from first instar to second, etc., but as little is known as yet as to the form of the function $\theta(t)$ these formulations will not be included here.

The egg and larval populations for a sufficiently small neighborhood, $T > t_3$

When an egg hatches, the larva is at first comparatively inactive, and as the only material lost during the transformation from egg to first instar larva is the minute amount which makes up the shell, during a sufficiently small neighborhood, $T > t_3$, first instar larvae may be thought of as eggs. In this case it will at once be apparent that the sum of the two populations, eggs and first instar larvae, will be equal to what the egg population alone would have been, in the absence of hatching, with the exception of those eggs which entirely pass out of existence owing to natural mortality at hatching. That is to say, if $\bar{N}_2$ be the value which the egg population would have reached at a time T, in the absence of hatching, and if N_2 and N_3 are the actual numbers of eggs and first instar larvae, at any such time,

$$N_2 - (1 - U_3)Z_3 = N_2 + N_3 \tag{67}$$

If now, as is the case, $(1 - U_3)$ be small, for a short time after hatching commences, two situations may arise:

(a) The egg population has reached a point of quasi-equilibrium, *i.e.*, is oscillating between the greatest integral number less than ξ and the least integer greater than ξ (see Equation 30). In this case as $N'_3 > 0$ when $T > t_3$, N'_2 will become negative as soon as $T > t_3$.

(b) Such a condition of quasi-equilibrium has not been reached by the egg population when $T = t_3$. In this case, the egg population will continue to increase until such a value is reached that

$$ReN_{11} = Z_3 + \sum_{i=3}^{i=k} C'_{i2} + C'_{11\,2}, \tag{68}$$

where k is the subscript for the oldest larva present at the time that the value of N_2 in Equation 68 is reached. After this time, the egg population decreases. Inactivity of first instar larvae is not necessary to the truth of the above expression however.

It seems hardly necessary to point out that immediately subsequent to $T = t_3$, the expression

$$U_3Z_3 > \sum_{i=4}^{i=k} C'_{i3} + C'_{11\,3}$$

must be satisfied if the larval population is ever to come into existence at all.

The question might now be asked as to what would occur should transformation to second instar larvae, (subscript 4) not take place. So far the writer has not been able to determine this point mathematically, but it would seem that there are two possibilities:

(c) The egg and larval populations come into stable equilibrium, in which the rate of egg transformation to first instar larvae $U_3(Z_3)$ at all times balances

the larval loss due to natural death, and due to the eating of larvae by larvae and adults, and in which Z_3, and the eating of eggs, and the death of eggs are together equal to $R\epsilon N_{11}$. That is, N_2' and N_3' are zero.

(d) The egg and first instar larval populations enter into an infinite series of "cyclical" changes, where by "cyclical" it is not necessarily meant that each cycle is identical with the preceding one, but is nevertheless of the same general form.

It is felt, lacking rigorous mathematical proof, that (c) can be ruled out for the following reason. At any time T the rate of production of first instar larvae is a function of all the past history of the egg and larval populations during a period of time from $T-\Gamma_2$ to T, that is to say,

$$U_3Z_3 = U_3\left[R\epsilon N_{11} - \int_{T-\Gamma_2}^{T} \theta(t)\left(\frac{C'_{3.2}+C'_{11.2}}{N_2}\right)dt\right], \text{ etc.} \tag{69}$$

Suppose that N_2 and N_3 have just arrived at such a pair of values that

$$U_3Z_3 = C'_{3.3}+C'_{11.3}, \tag{70}$$

i.e., the equilibrium mentioned under case (a) is attained. Let T receive a finite increment ΔT, then,

$$U_3Z_3 = U_3\left[R\epsilon N_{11} - \int_{T+\Delta T-\Gamma_2}^{T+\Delta T} \theta(t)\left(\frac{C'_{3.2}+C'_{11.2}}{N_2}\right)dt\right]. \tag{71}$$

Then if U_3Z_3 still equals $C'_{3.3}+C'_{11.3}$, N_2 and N_3 must have had the values mentioned above throughout the interval $T-\Gamma_2$ to T, whence the above equilibrium cannot be maintained for a finite time unless it has already been maintained for a period Γ_2. Since N_2 and N_3 are not at these equilibrium values when $T=t_3$, they can never, on attaining them, remain at them for a finite time. It follows that where $Z_4=0$, *i.e.*, where no transformations to second instar larvae occur, the first instar larvae increase in numbers until

$$U_3Z_3 = C'_{3.3}+C'_{11.3},$$

and then, due to eating, decrease again. A second such maximum cannot occur again until a period of at least Γ_2 has passed, for not until then can the increase in egg population (resulting from a decrease in $C'_{3.2}$) have any major effect upon the larval population.

It is true, of course, that there will be a retardation in the rate of decrease of N_3 due to the increase in Z_3 with decrease in $C'_{3.2}$ but this cannot in general make $N_3'>0$.

Generally speaking, where $Z_4>0$, the above-mentioned maximum value of the first instar larvae is not attained, owing to the reduction caused by Z_4.

It is believed that the other larval instars increase in numbers, tending towards analogous maxima, and in general, decrease in numbers before reaching them, for analogous reasons.

Distribution by ages in the total larval population

During actual counts of larval populations which had been running for some time, it was noticed that there was an increase in the proportion of older larvae, even after the time when larvae of all stages were present. That is to say, with older populations the distribution by ages tends to become increasingly skew in the direction of the greatest age. The explanation of this phenomenon from the theory thus far developed is comparatively simple.

Consider a group of first instar larvae hatching from eggs during a period $t_3 < T < t_3 + \Delta t$, where Δt is some small increment of time. By reason of the postulate that $C'_{i.j} = 0$, $i < j$ except $j = 9$, all through their lives this small group is subjected to an "eating force" of only $C'_{j.j} + C'_{11.j}$, where j is the subscript indicative of the instar in which the group exists at any time. On the other hand, a similar group hatching out over a period of equal duration, but situated later in time, when there are perhaps larvae of the second and third instars present, will be subject to much greater eating, namely, $C'_{(j+2).j} + C'_{(j+1).j} + C'_{j.j} + C'_{11.j}$. Not only this, but the group will be smaller to start with, since it is easily seen that in populations of the type described in this paper $\frac{\partial Z_i}{\partial T} < 0$ where i is the subscript of any larval instar. Thus, theoretically at least, the first larva hatching promptly at $T = t_3$ will always pupate, provided it is not eaten by an adult, and does not die from some natural cause. Obviously then, with the passage of time, all but the older larvae tend to disappear, and the age distribution is skewed in the direction of the greatest age.

Pupal populations

In general it may be said that the pupal populations tend, as do the larval populations, to reach a maximum value, at which the increase due to transformation from sixth instar larvae (subscript 8) just balances the loss by natural death and by eating, but do not attain this value by reason of transformation to the adult form.

Adult populations, $T > t_{10}$

The equation descriptive of the growth of the adult population is in general far simpler than those descriptive of the growths of the egg, larval and pupal populations owing to the fact that $C'_{i.10}, C_{i.11} = 0$.

Therefore, the only negative term will be one to account for the slow and gradual death of adults by reason of sheer accident and infirmity. This term may be written in the form $\overline{K}\ (T - t_{11})$ where $\overline{K}$ is a constant.

What then limits the size of the adult population? The equation may be written as

$$N_{11} = Z_{11} - \overline{K}(T - t_{11}), \tag{72}$$

whence it is at once seen that the limiting factor, since $\overline{K}$ is very small, is a diminution in Z_{11}, the rate of transformation from immature to mature adults. Thus, if $\overline{K}$ be considered as practically negligible, N_{11} will increase until $Z_{11} = 0$ which practically, as $C'_{i.10} \doteq 0$, means $Z_{10} = 0$.

An interesting point arises here. Suppose that Y adults, mature and immature, are sufficient to reduce and hold the pupal population practically at zero, so that N'_{10} is zero. By reason of the fact that some time is necessary to eliminate the pupal population in existence at $T=t_{10}$ the adult population generally rises above the value Y which would obtain under any particular set of environmental conditions (temperature, flour mass, etc.) As this large adult population cannot reduce the pupal population to less than zero, it feeds upon flour, eggs, and such larvae as emerge from time to time, and in the meantime dwindles slowly away (see Figs. 4 and 5) by reason of the second term in Equation 72, to approach the value Y from above.

Owing to unavoidable failure in the mechanical equipment used to maintain constant temperatures, no populations were grown for a sufficient length of time to determine what would happen beyond this point, but in the case of some populations in which the number of adults was accidentally greatly reduced, a new cycle analogous with those mentioned on page 660 commenced, the adult population rising again to a value in excess of Y. It is believed that a periodic fluctuation with a period of $t_e + \dfrac{\overline{N}_{11} - Y}{\overline{K}}$ would be set up, where $\overline{N}_{11}$ is the maximum number of adults reached under any particular set of conditions, and t_e is the time from t_2 to the time of reaching the maximum number of adults.

It might also be asked how hatching of eggs can fail to occur, as it at times does, when subsequent to $T=S$ there are perhaps 1000 eggs present. The answer to this lies in the fact that Z_3 is dependent not only upon the number of eggs, but also on the rates of consumption of the same throughout their life as eggs. In a case such as the above, the rates of consumption are such that the probability that an egg will be found and eaten in a time less than Γ_2 is 1. Hence no eggs survive long enough to hatch, even though they are very numerous. Such a condition is of course unstable, in that a diminution of larvae or other predators will cause an immediate increase in the numbers of first instar larvae.

The Protective Influence of One Form on Another, in the Matter of Eating

Consider the addition of a new form to the system at any time. It will be apparent that if this new form is edible, it must bear some of the depredations of the predators, and for that reason must diminish to some extent the rate at which all other edible forms are consumed. The mechanism of this, mathematically, is quite simple, as it operates through the fact that the addition of a new edible form adds another Q to the denominator of all the C''s already in operation, and since the added quantity is real and positive, the C''s are thereby diminished. If it should occur that the added form is predatory as well as edible, the protection offered may be more than offset by the additional predatory influence. This will of course depend on the relative value of the new C' introduced and the diminution in those already present.

This matter will be referred to more extensively in Part 3.

Part 3

Discussion of Experimental Data

In Tables X, XI, XII and XIII are tabulated the actual population counts from duplicate experiments at four temperatures, namely, 17°, 22°, 27°, and 32°C. all at 75% relative humidity. (This was somewhat exceeded in the cases of the two populations at the lower temperatures owing to unavoidable condensation on the cooling coils in the controlled cabinets.) The duplicate experiments are denoted as (A) and (B). The mean values from (A) and (B) are also shown.

These means are plotted as Figs. 2, 4, 6 and 8, while Figs. 3, 5, 7 and 9 show curves drawn on a basis of the actual data, but with modifications made in an endeavor to replace information lost by reason of the relative infrequency of the counts. These changes have been made on the basis of the information gained from the theory developed in Part 2. Care has been taken to make

TABLE X*

Population series in whole wheat flour, 32 gm., at 17°C. and 75% relative humidity

Time, days	Eggs (A)	Eggs (B)	Eggs Mean	Larv. (A)	Larv. (B)	Larv. Mean	Ad. (A)	Ad. (B)	Ad. Mean	Tot. (A)	Tot. (B)	Tot. Mean
0	16						16	16	16	16	16	16
10	41	24	37				16	16	16	57	40	48
20	61	47	54				16	16	16	77	63	70
30	102	93	97				16	16	16	118	109	113
40	134	112	123				16	16	16	150	128	139
50	162	141	152				16	16	16	178	157	167
60	186	160	173	2		1	16	16	16	204	176	190
70	178	153	165	1		.5	16	16	16	195	168	182
80	219	143	181	3	2	2.5	16	16	16	248	161	204
90	245	130	187	5	0	2.5	16	15	15.5	266	145	205
100	253	149	201	5	3	4	16	15	15.5	274	167	220
110	249	168	208	4	0	2	16	15	15.5	269	183	226
120	214	169	191	13	1	7	16	15	15.5	243	185	191
130	222	168	195	13	1	7	16	15	15.5	251	184	217
140	309	189	249	14	0	7	15	15	15	338	204	271
150	275	203	239	12	.0	6	15	15	15	302	208	255
160	190	186	188	8	G	4	15	15	15	213	201	207
170	212	213	212	8	5	6	15	15	15	235	243	239
180	203	220	211	6	5	5	15	15	15	224	240	232
190	243	265	254	8	3	5	15	15	15	266	283	274
200	258	230	244	15	9	12	15	15	15	288	254	271
210	219	245	232	16	19	17	15	15	15	250	269	259
220	210	224	217	31	39	35	15	15	15	256	278	267
230	261	233	247	32	32	32	15	15	15	308	280	294
240	221	201	211	8	57	32	15	15	15	244	273	258
250	229	190	209	20	82	51	15	15	15	304	287	295
260†	...	...	...	...	...	....	...	...	....	..	...	...
270	234	174	204	42	138	90	15	15	15	291	327	309
280	219	149	184	40	95	67	15	15	15	274	259	266

* *The writer is indebted to Dr. R. N. Chapman for the data in this table.*

† *Count missed on this day.*

Note:—*No pupation occurred at 17°C.*

only such modifications as could be found homologously at all temperatures with the exception of 17°C., in which case, owing to the great value of Γ_2 only the earliest stages of the population growth are manifest.

As soon as T becomes greater than $t_2=0$, eggs commence to accumulate according to Equation 29, and as has been shown, tend towards the limiting value ξ, at which point they are found and eaten as rapidly as they are laid. At 17°C., owing to the high value of Γ_2, this condition is practically attained, but as at times, a few eggs do hatch, in the region of 60 to 70 days, Fig. 3,

$$N'_2 = R\epsilon N_{11} - Z_3 - C'_{3.2} - C'_{11.2} < 0. \tag{73}$$

At 22°, 27°, and 32°C. this equilibrium value ξ is not reached owing to the reduced value of Γ_2, and at p_1 the egg population reaches a maximum as described in Equation 68, and then decreases owing to hatching to form first instar larvae.

Thus, the population at 17°C. is an example of Case (a) page 659 while the other three populations illustrate Case (b), page 659.

TABLE XI*

POPULATION SERIES IN WHOLE WHEAT FLOUR, 32 GM., AT 22°C. AND 75% RELATIVE HUMIDITY

Time, days	Eggs (A)	Eggs (B)	Eggs Mean	Larv. (A)	Larv. (B)	Larv. Mean	Pup. (A)	Pup. (B)	Pup. Mean	Ad. (A)	Ad. (B)	Ad. Mean	Tot. (A)	Tot. (B)	Tot. Mean
0										16	16	16	16	16	16
10	196	222	209	11	12	11				16	16	16	223	250	236
20	338	314	326	79	661	70				16	16	16	433	391	414
30	399	383	391	210	131	170				16	16	16	625	530	577
40	371	398	384	323	219	271	0	0	0	16	16	16	710	633	671
50	287	281	284	315	204	259	1	3	2	16	16	16	619	504	561
60	225	186	205	365	326	345	7	9	8	16	19	17	613	440	526
70	200	149	174	372	248	310	22	22	22	19	26	22	613	445	529
80	132	118	125	297	153	225	97	45	71	27	35	31	553	356	454
90	79	124	101	175	176	175	93	81	87	76	59	62	423	440	431
100	106	159	132	92	129	110	57	74	65	114	83	98	369	445	407
110	161	243	202	47	82	64	10	58	34	136	124	130	354	507	430
120	157	351	254	45	83	64	2	37	19	130	142	136	334	613	473
130	128	347	237	69	176	122	2	9	5	120	148	134	319	680	499
140	151	325	238	72	131	101	0	2	1	109	140	124	325	598	461
150	148	235	191	77	155	116	0	0	0	101	137	119	326	527	426
160	87	158	122	67	162	114	0	0	0	92	128	110	246	448	347
170	250	382	316	37	167	102	0	0	0	87	120	103	374	669	521
180	621	610	615	52	122	87	0	0	0	86	119	102	759	851	805
190	487	336	411	207	201	204	1	1	1	80	121	100	774	659	716
200	377	224	300	248	182	215	0	1	1	76	118	97	701	525	603
210	224	134	179	346	201	273	0	5	2	71	114	92	641	454	547
220	144	65	104	328	184	256	0	9	4	69	106	87	541	364	447
230	83	49	66	307	148	227	6	2	4	63	88	75	409	287	348
240	50	37	43	279	96	187	13	0	6	62	80	71	404	213	308
250	54	34	44	263	59	161	7	1	4	60	64	62	384	158	271
260	268	158	218	155	23	89	0	0	0	56	55	55	479	236	357
270	284	554	419	230	44	137	0	0	0	55	48	51	669	646	657
280	521	580	550	300	202	251	0	0	0	55	46	50	876	828	852

* *The writer is indebted to Dr. R. N. Chapman for the data in this table.*

The writer confesses inability to explain the sudden fall in larval population at p_2 in the case of the population grown at 22°C. Lacking knowledge of the value of the Γ's for 22°C., it is not possible to determine whether this is an inherent peculiarity of the population growth or whether it is due to faulty technique. It is possible that it is due to relatively great activity on the part of sixth instar larvae at this temperature, but this is hardly in accord with observations made of such larvae. Moreover, such activity would manifest itself in an even sharper decline in egg population. Again, had such a decrease in the larval population actually occurred, it would have been reflected in a similar drop in pupal population at a time Γ_8 later. As there is no evidence of this, beyond the fact that the pupal curve seems somewhat bluntly truncated at 90 days, the time of the expected discrepancy, it is assumed that it is due to an error in count of an even hundred in "B" at 50 days, the finely waved line indicating the appropriate correction.

T now increases to approach and exceed t_9, and as pupae make their appearance,

$$N'_9 = U_9 Z_9 - \sum_{i=3}^{i=8} C'_{i,9} - C'_{11,9} > 0. \tag{74}$$

TABLE XII*

POPULATION SERIES IN WHOLE WHEAT FLOUR, 32 GM., AT 27°C. AND 75% RELATIVE HUMIDITY

Time, days	Eggs (A)	Eggs (B)	Eggs Mean	Larv. (A)	Larv. (B)	Larv. Mean	Pup. (A)	Pup. (B)	Pup. Mean	Ad. (A)	Ad. (B)	Ad. Mean	Tot. (A)	Tot. (B)	Tot. Mean
0										16	16	16	16	16	16
10	363	383	373	18	51	34				15	17	16	396	451	423
20	350	299	324	319	316	317		1	1	14	17	15	669	633	651
25	213	227	220	377	388	382	2	6	4	13	16	14	605	637	621
30	149	143	146	365	403	384	7	14	10	13	17	15	534	577	555
35	259	261	260	309	308	308	56	73	64	19	24	21	643	666	654
40	202	225	213	261	288	274	171	171	171	26	46	36	660	730	695
46	331	229	280	157	183	170	138	133	135	161	173	167	779	718	748
50	341	218	279	128	159	143	69	90	79	229	236	232	767	703	735
55	334	332	333	144	142	143	26	27	26	272	292	282	776	793	784
60	328	331	329	104	137	120	118	21	19	285	304	294	735	793	789
70	428	327	377	41	87	64	24	39	31	299	333	316	792	786	789
80	522	492	507	12	30	21	17	27	22	308	356	332	859	905	882
90	706	869	782	10	3	6	3	27	15	303	365	334	1022	1264	1143
100	824	1040	932	49	36	42	4	1	2	317	371	344	1194	1448	1321
110	812	750	781	73	109	91	1	1	1	297	367	332	1183	1227	1305
120	509	615	562	221	157	189	15	15	15	316	374	345	961	1097	1029
130	388	460	424	107	100	103	27	40	33	321	384	352	843	984	913
140	491	717	604	92	64	78	16	35	25	325	390	357	924	1206	1065
150	323	681	502	75	47	61	18	14	16	323	405	364	739	1147	943
160	216	274	245	73	98	85	14	8	11	332	416	374	635	796	715
170	372	446	409	59	74	66	2	12	7	326	412	369	759	944	851
180	138	110	124	30	57	43	0	13	6	319	401	360	487	581	534
190†	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....

* *The writer is indebted to Dr. R. N. Chapman for the data in this table.*

† *Population accidentally killed at 190 days by escape of ammonia from refrigeration equipment.*

This pupal population tends to increase to such a point that,

$$U_9Z_9=\sum_{i=3}^{i=8}C'_{i.9}-C'_{11.9}, \tag{75}$$

but by reason of transformation to immature adults, arrives only at the maximum at p_7 where

$$U_9Z_9=\sum_{i=3}^{i=8}C'_{i.9}-C'_{11.9}-C'_{10.9}-Z_{10}. \tag{76}$$

The larval population continues to increase subsequent to $T=t_9$, for a short period, reaching a maximum at p_3 where

$$N'_L=U_3Z_3-Z_9-\sum_{i=j}^{i=11}\sum_{j=3}^{i=8}C_{i.j}=0^* \tag{77}$$

Subsequent to $T=t_9$ and also to the time of p_3, the egg population continues to fall, to reach a minimum where,

$$N'_2=R\epsilon N_{11}-Z_3-\sum_{i=3}^{i=8}C'_{i.2}-C'_{11.2}\,. \tag{78}$$

TABLE XIII†

POPULATION SERIES IN WHOLE WHEAT FLOUR, 32 GM., AT 32°C. AND 75% RELATIVE HUMIDITY

Time, days	Eggs (A)	Eggs (B)	Eggs Mean	Larv. (A)	Larv. (B)	Larv. Mean	Pup. (A)	Pup. (B)	Pup. Mean	Ad. (A)	Ad. (B)	Ad. Mean	Tot. (A)	Tot (B)	Tot. Mean
0										16	16	16	16	16	16
10	344	366	355	182	213	197	0	0	0	15	16	15	541	595	568
20	115	100	107	423	457	440	3	0	1	14	15	14	555	572	563
25	129	106	117	274	281	277	110	131	120	15	14	14	528	532	530
30	127	172	149	122	130	126	216	241	228	89	96	92	554	639	596
35	446	270	358	93	118	105	117	87	102	231	307	264	887	882	884
40	358	257	307	56	83	69	26	19	22	343	358	350	783	737	760
46	381	352	366	46	67	56	3	13	8	352	366	359	782	798	790
50	390	375	382	44	40	42	2	7	4	357	368	362	793	790	791
60	465	456	460	4	7	5	4	5	5	376	371	373	849	839	844
70	711	669	690	4	7	7	0	0	0	362	363	362	1077	1036	1056
80	837	687	762	17	6	11	0	1	1	358	371	364	1212	1065	1138
90	993	838	915	13	5	9	0	0	0	361	362	361	1367	1205	1286
100	940	925	932	11	6	8	2	1	1	353	361	357	1306	1293	1299
110	1263	963	1113	55	35	45	1	1	1	341	359	350	1658	1338	1498
120	744	559	651	139	42	90	3	2	2	341	360	350	1227	963	1095
130	448	738	593	106	33	119	10	2	6	332	355	343	896	1128	1013
140	765	906	835	58	25	41	14	9	12	315	335	325	1152	1275	1213
150	890	573	731	37	134	85	1	1	1	315	323	319	1243	1031	1137
160	653	280	466	69	91	80	0	22	11	307	288	297	1026	681	853
170	614	497	555	87	23	55	0	2	1	284	260	272	985	782	883
180	919	1032	975	122	260	191	0	0	0	279	254	266	1320	1546	1433
190	515	190	352	151	333	242	2	0	1	257	225	241	925	748	836
200	506	187	346	106	238	172	5	67	36	214	204	209	831	696	763

† *The writer is indebted to Dr. R. N. Chapman for the data in this table.*
NOTE:—*Population accidentally destroyed by failure of temperature control, at 203 days.*
* *Note that $N_{10}=0$ and that $C'_{9.j}=0$.*

and then, by reason of reduction in the numbers of the highly predatory sixth instar larvae (subscript 8), and by reason of the protective influence of the edible pupae (see page 662), acting as an alternative food for the remaining predators, N'_2 becomes greater than zero, and the egg population again increases.

In the case of the population grown at 22°C. there is an additional refutation of the idea that sixth instar larvae grown at 22°C. are highly predatory as the diminution of their numbers does not decrease the rate of eating of eggs sufficiently to cause a great rise in the egg population. Possibly such a rise did occur, and was missed on account of the comparative infrequency of counts. It is an unavoidable misfortune that no count was made at 65 days.

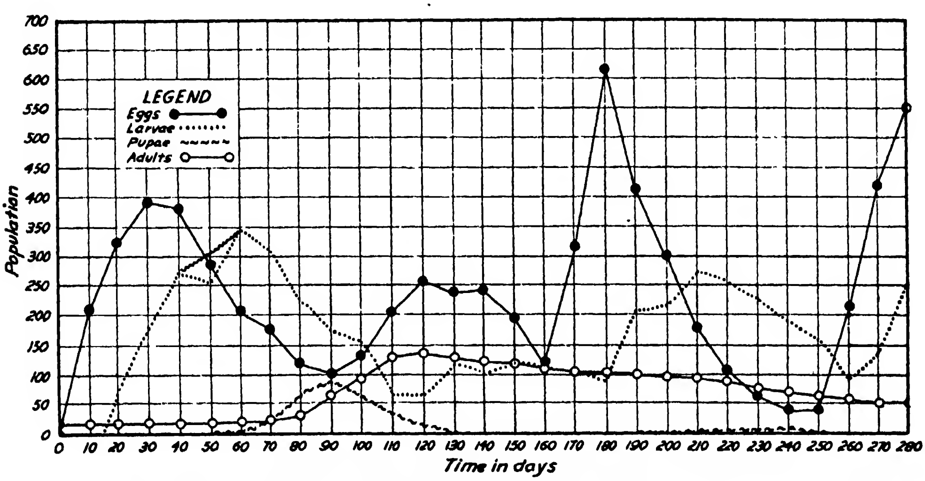

FIG. 4. *Population growth of T. confusum, Duv. at 22°C., mean of (A) and (B) populations.*

T now increases to approach and exceed t_{10} when immature adults (subscript 10) begin to come into existence. For a short time subsequent to t_{10} the egg population continues to increase, until with increase in N_{10} a maximum is reached at p_5 where,

$$N'_2 = R\epsilon N_{11} - Z_3 - \sum_{i=3}^{i=8} C'_{i.2} - C'_{10\cdot 2} - C'_{11.2}. \tag{79}$$

Subsequent to this time, for the same reasons, N'_2 becomes less than zero, and the egg population falls to reach a minimum at p_6 which will be discussed later.

In the meantime, subsequent to t_{10}, the pupal population continues to increase until a maximum is reached at p_7, due to increase in Z_{10}. The writer is unable to say whether Z_{10} is increasing or decreasing at this time. However, at p_7, Equation 76 must hold. Immediately subsequent to p_7, N'_9 becomes negative by a continuation of the reasoning set forth in the above paragraph.

During the period subsequent to t_9, the protective influence of the pupae and the progressive diminution in Z_{10} produce a slight increase in the rate of increase of larvae in general.

To return to a consideration of p_6: the reversal of the sign of the derivative N'_2 is due to transformation of the predatory but non-egg-laying immature adults into predatory but egg-laying mature adults. Thus by the operation

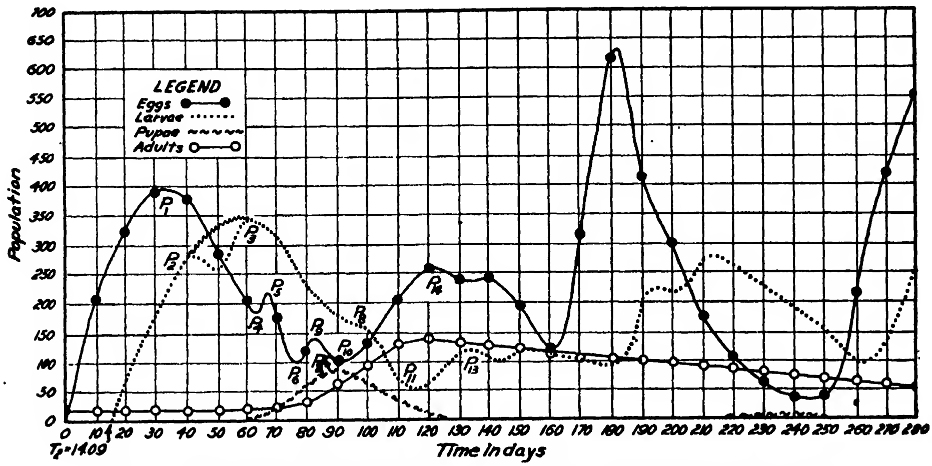

FIG. 5. *Smoothed curves for population growth of T. confusum, Duv. at 22°C.*

of Z_{11}, the function descriptive of the rate of this transformation, N_{11} is greatly increased, and thus $R\epsilon N_{11}$ is increased. This results in an increase in N'_2, so that it becomes greater than zero, and the egg population rises to reach a maximum again at p_9.

This further arrest in the increase in the number of eggs is due to the withdrawal of pupae as an alternative food for the predators subsequent to p_6. A similar reduction in the numbers of larvae is seen immediately subsequent to p_8.

Subsequent to p_9, by the continued operation of this factor, N'_2 again becomes negative, and the egg population falls to another minimum at p_{10}, where, by reason of the continued increase in N_{11}, the sign is again reversed.

Subsequent to p_{10}, the egg production increases

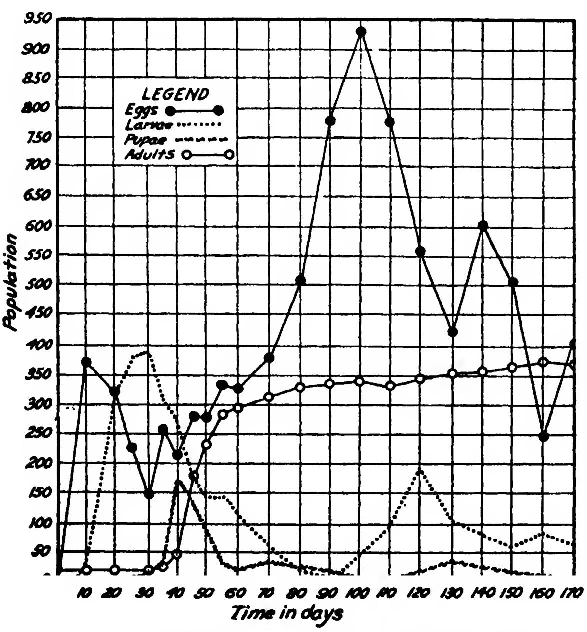

FIG. 6. *Population growth of T. confusum, Duv. at 27°C., mean of (A) and (B) populations.*

enormously, and here again the protective influence of an alternative food is seen, as before any sensible increase in the rate of hatching can take place, an increase in the larval population is seen at p_{11}.

This effect upon the larvae is in turn reflected in a slight reduction in N'_2 as seen at p_{12} (p_{12} is not clearly defined at 22°C. owing to slight larval activity at this temperature) following which the egg population continues to increase.

During this enormous rise in the egg population, although C''s of the form $C'_{i.2}$ are very great, not all eggs can be consumed by the adults and the comparatively small larval populations before the eggs have reached an age Γ_2. Hence some hatching does occur to cause an increase in the larval population at p_{13}. This increase in turn so augments the predatory population as to again reverse the sign of N'_2 and decrease the egg population subsequent to p_{14}.

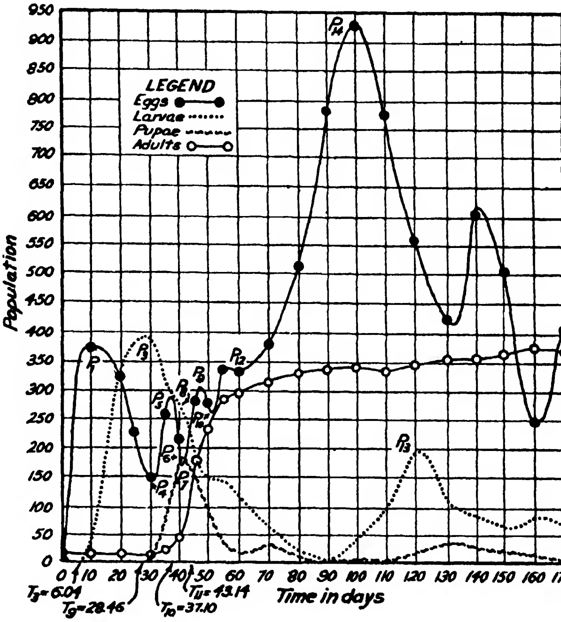

FIG. 7. *Smoothed curves for population growth of T. confusum, Duv. at 27°C.*

This periodic fluctuation in the numbers of eggs and larvae is believed to continue indefinitely. In the case of two populations grown at the University of Minnesota, owing to an infestation of intestinal parasites (*Gregarina*), pupae were unable to transform to immature adults, and slowly withered away a few days after pupation. In these two cases the egg and larval populations fluctuated regularly and repeatedly, with a period roughly equal to $\sum_{i=2}^{i=8} \Gamma_i$ while the adult population slowly dwindled away as explained on page 661. In such a case, the population as a whole would in time die out completely, unless the parasites decreased sufficiently to allow a few new adults to emerge. This special case will be described in a later paper.

Meanwhile, the adult population has risen to a maximum where $Z_{10}=0$ and from then on, slowly declines, while Z_{10} remains practically zero, as explained on page 661. It will be noticed that as N_{11} decreases, the amplitude of the pupal fluctuations becomes greater. While N_{11} remains greater than Y (see page 662) few or no pupae emerge, despite the brief increases in the pupal population, but as soon as N_{11} falls below Y, as explained above, a new production of adults begins the cycle again.

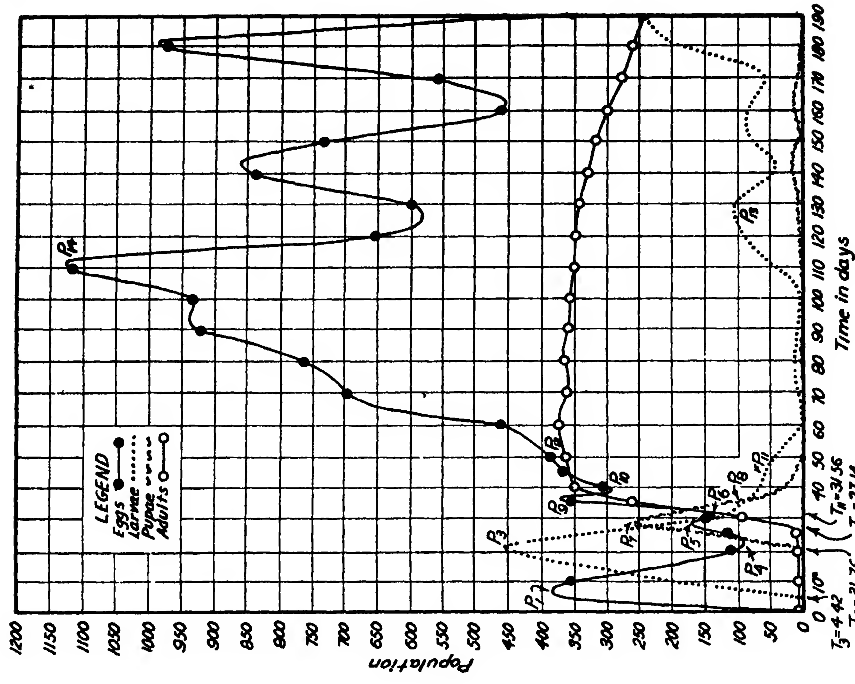

FIG. 9. Smoothed curves for population growth of *T. confusum*, Duv. at 32°C.

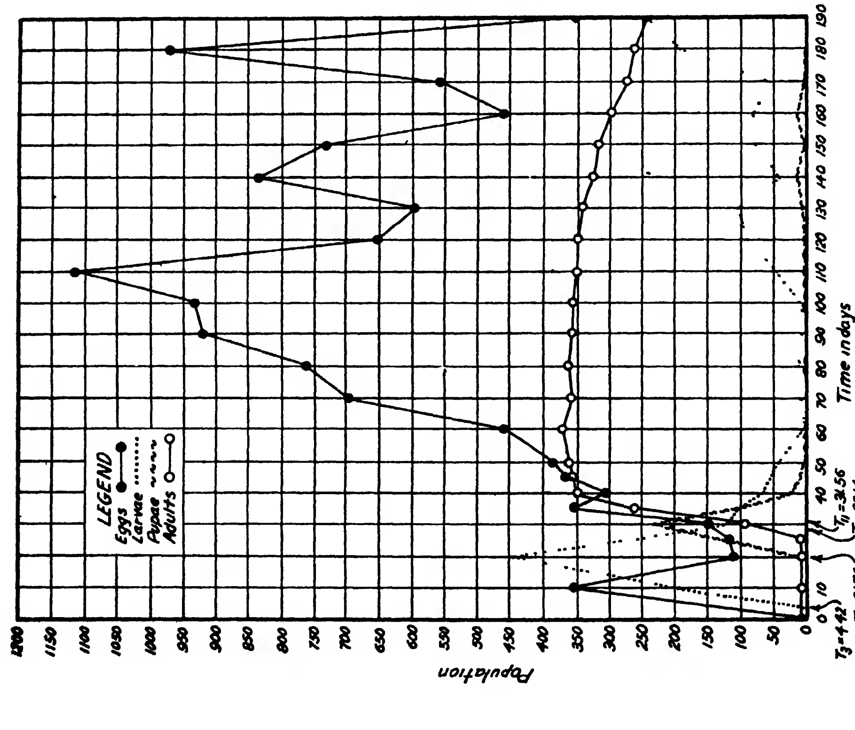

FIG. 8. Population growth of *T. confusum*, Duv. at 32°C., mean of (A) and (B) populations

The writer does not believe that two identical cycles can ever follow one another in finite time, as the population at any instant is a function of its whole past history. It is highly probable however that succeeding cycles approach some fixed form, reaching the same after an infinite time.

Acknowledgment

The writer wishes to express his indebtedness to Dr. R. N. Chapman for the original suggestion of this problem, for the use of a great deal of biological data, and for his interest and encouragement in the working out of the problem, both at Minnesota and in Honolulu.

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References

1. Allee, W. C. Animal Aggregations. Univ. of Chicago Press. 1931.
2. Bailey, V. A. The Interaction between hosts and parasites. Quart. J. of Math. Oxford Series 2: 1931.
3. Brindley, T. A. The growth and development of *Ephestia Kuehniella* Zeller (Lepidoptera) and *Tribolium confusum* Duv. (Coleoptera) under controlled conditions of temperature and relative humidity. Ann. Ent. Soc. Amer. 23: 741-757. 1930.
4. Chapman, R. N. The confused flour beetle. Annual Report No. 17. State Entomologist of Minn. 73-94. 1918.
5. Chapman, R. N. Insects infesting stored food products. Minn. Agric. Exp. Stn. Bull. 198: 45-50. 1921.
6 Chapman, R. N. Inhibiting the process of metamorphosis in the Confused Flour Beetle. Journ. Exp. Zool. 45: 293-299. 1926.
7. Chapman, R. N. Quantitative analysis of environmental factors. Ecology, 9: 111-122. 1928.
8. Gause, G. F. The influence of ecological factors on size of population. Amer. Nat. 45: 70-76. 1931.
9. Holdaway, F. G. Nutritional status and sex determination. Nature, 126: 131. 1930.
10. Loeb, L. B. Kinetic theory of gases. McGraw Hill, New York. 1927.
11. Park, T. Studies in population physiology. The relation of numbers to initial population growth in the flour beetle. *Tribolium confusum* Duv. Ecology, 13: 172-181. 1932.
12. Sweetman, M. D. and Palmer, L. S. Insects as test animals in vitamin research. I. Vitamin requirements of *Tribolium confusum* Duv. J. Biol. Chem. 77: 33-52. 1928.
13. Volterra, V. Variazioni e fluttuazioni del numero d'individui in specie animali conviventi. Accad. Naz. d. Lincei. Classe d. Sci. fisiche. mat. e nat. Ser. VI, vol. II fasc. III. 31-112. 1926.
14. Volterra, V. Variazioni e fluttuazioni del numero d'individui in specie animali conviventi. R. Com. Tal. Ital. N. 599. 1927.
15. Volterra, V. Leçons sur la théorie mathématique de la lutte pour la vie. Cahier. Sci. fasc. VII. 1931.